



**INÊS ALVES DOS  
SANTOS TEIXEIRA  
GOMES**

**CONECTIVIDADE E AVALIAÇÃO BIOLÓGICA;  
FERRAMENTAS PARA AVALIAR A COERÊNCIA  
ECOLÓGICA DE REDES DE ÁREAS MARINHAS  
PROTEGIDAS**

**CONNECTIVITY AND BIOLOGICAL VALUATION;  
TOOLS TO ASSESS THE ECOLOGICAL  
COHERENCE OF NETWORKS OF MARINE  
PROTECTED AREAS**



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OF MARINE PROTECTED AREAS**

Tese apresentada à Universidade de Aveiro para cumprimento dos requisitos necessários à obtenção do grau de Doutor em Ciências do Mar, realizada sob a orientação científica do Professor Doutor Henrique José de Barros Brito Queiroga, Professor Auxiliar do Departamento de Biologia da Universidade de Aveiro e co-orientação da Professora Doutora Ann Vanreusel, Professora Catedrática do Departamento de Biologia da Universidade de Ghent, Bélgica.

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*“The fun factor isn’t essential to creative work, (...) but sometimes when we’re playing around with ideas and laughing, we’re most open to new thoughts. In all creative work, there may be frustrations, problems, and dead ends along the way. (...) However, there’s always profound pleasure at some point, and a deep sense of satisfaction from “getting it right.”*

Ken Robinson, *The Element: How Finding Your Passion Changes Everything*.

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.

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## palavras-chave

Coerência Ecológica; Redes de Áreas Marinhas Protegidas; Conectividade; Representatividade; Etiquetas naturais; Veículos aéreos não tripulados; Modelos Biofísicos; Avaliação Biológica Marinha.

## resumo

A pressão internacional para encontrar abordagens eficazes baseadas no ecossistema marinho, de maneira a evitar, reduzir e compensar o impacto das atividades humanas na saúde global dos oceanos, está a aumentar. As principais políticas marítimas europeias e outras agências internacionais, defendem que para proteger efetivamente o meio marinho, é necessário estabelecer redes ecologicamente coerentes de áreas marinhas protegidas (AMPs).

Atingir a coerência ecológica é, no entanto, uma tarefa complexa dada a magnitude do conceito e a falta de definições operacionais objectivas. Deste modo, avaliar a coerência ecológica requer abordagens múltiplas de modo a analisar a localização, tamanho e espaçamento entre áreas protegidas já seleccionadas, mas também para inferir sobre a designação de novos locais, melhorando a performance da rede. O objetivo desta tese é desenvolver e usar diferentes ferramentas para apoiar e promover a avaliação da coerência ecológica das redes de AMPs, com foco em Portugal continental.

Este trabalho utilizou três métodos complementares usando o mexilhão *Mytilus galloprovincialis* como espécie-modelo, para estimar conectividade larvar ao longo da costa oeste portuguesa. O primeiro método retrata o uso de etiquetas geoquímicas naturais para fazer o rastreio da origem natal de mexilhões, quantificando padrões de conectividade demográfica (trajetórias de dispersão e distâncias). Concentrou-se nas AMPs da Arrábida e Berlengas, salientando a importância das populações que servem como fonte de indivíduos para dispersão e auto-recrutamento dentro das AMPs. O segundo método resultou da necessidade de quantificar a cobertura de mexilhão no intermareal rochoso, para aplicar posteriormente em modelos de dispersão de larga escala. O uso de imagens aéreas de baixa altitude e de alta resolução, modelos de superfície tridimensional e de observações in situ, provou ser uma ferramenta viável para monitorizar ecossistemas intermareais numa escala espacial ecologicamente relevante. O efeito de variáveis ambientais (complexidade do substrato e exposição às ondas) na densidade e tamanho do mexilhão foi também investigado. A densidade máxima de mexilhão ocorreu com valores intermédios de exposição às ondas no Inverno enquanto que o menor tamanho dos mexilhões estava relacionados com altos valores de exposição às ondas.

## resumo (cont.)

A combinação das estimativas empíricas de conectividade, com parâmetros demográficos do mexilhão (fertilidade, comportamento larval, mortalidade) permitiram a validação de um modelo numérico biofísico de larga escala, no terceiro estudo. Este modelo combinou informações oceanográficas, demográficas e de biologia larvar, para simular a dispersão de larvas de *M. galloprovincialis* ao longo da costa oeste da península Ibérica. O modelo incorporou e simulou também a incerteza associada à atribuição da origem das populações relativa ao método geoquímico, atingindo altos níveis de correspondência entre as estimativas independentes de conectividade a uma pequena escala espacial. Este modelo validado pode ser usado no futuro para investigar dinâmicas metapopulacionais com aplicações em estratégias de gestão de AMPs.

Finalmente, realizou-se uma abordagem ecológica para valorizar a biodiversidade marinha ao longo da plataforma continental portuguesa. Este protocolo de avaliação biológica integrou informações biológicas e ecológicas espaciais, para diversos componentes do ecossistema, num mapa global de valor biológico marinho, destacando hotspots de significância ecológica. Este estudo avaliou também em que medida as zonas de alto valor biológico estavam incluído nas actuais e futuras áreas protegidas da rede Natura 2000 em Portugal.

Ao integrar a informação das vias de conectividade marinha, com uma ampla avaliação biológica da costa portuguesa, esta tese procura contribuir para os critérios de avaliação de redes de AMPs e dar um passo em frente no sentido de o tornar aplicável ao panorama português.

## keywords

Ecological-Coherence; Marine Protected Areas; Networks; Connectivity; Representativity; Natural tags; Unmanned Aerial Vehicles; Biophysical modeling; Marine Biological Valuation.

## abstract

In order to avoid, reduce and offset the impact that human activities are having on the health of the world's oceans, the international demand for a successful ecosystem based approach to the marine environment is growing in popularity. Major European maritime policies and other international agencies advocate that, in order to effectively protect the marine environment, there is a need to establish ecologically coherent networks of marine protected areas (MPAs). Achieving ecological coherence is, however, a complex task given the magnitude of the concept and the lack of clear operational definitions. Therefore, assessing ecological coherence requires multiple approaches to analyse not only the location, size and spacing of protected sites already selected for protection, but also infer about new sites to enhance network effects. The aim of this thesis is to develop and use different tools to assist, support and help to promote the assessment of the ecological coherence of networks of MPAs, with a focus on continental Portugal.

Three complementary methods were applied using the mussel *Mytilus galloprovincialis* as a model species to estimate connectivity via larval dispersal along the Portuguese west coast. The first study described the use of natural geochemical tags to trace back natal origins of mussels and describe direct measures of demographic connectivity patterns (dispersal trajectories and distances). It focused in the Arrábida and Berlengas Marine Protected Areas (MPAs), and emphasized the significance of source and sink populations and self-recruitment within MPAs. The second study resulted from the need to quantify mussel bed coverage in order to apply the connectivity estimates into broader scale models of larval dispersal. The use of low altitude and high-resolution drone imagery, 3D surface models and ground based observations proved to be a viable and powerful tool for surveying intertidal ecosystems at an ecological relevant spatial scale. The effect of environmental variables (substrate complexity and wave exposure) on mussel density and size was also investigated. Maximum mussel density occurred at intermediate values of wave exposure in winter while smaller sized mussels prevailed at high values of wave exposure.

**abstract  
(cont.)**

The combination of the empirical connectivity estimates, with mussel demographic and biological parameters (reproductive output, larval behaviour, mortality) allowed the validation of a broad scale numerical biophysical model in the third study. This model combined oceanographic and demographic information with larval biology, and was used to simulate dispersal of *M. galloprovincialis* larvae along the Western Iberian coast. The biophysical model simulated and accounted for uncertainty of the geochemical method in the assignment of source populations and resulted in unprecedented levels of correspondence among independent connectivity estimates at small spatial scales. This fine-tuned model can be used in the future to investigate metapopulation dynamics with applications in MPA management strategies.

Finally, a quantitative-based ecological approach was used to value biodiversity along the Portuguese continental shelf. The marine biological valuation protocol summarized and combined existing biological and ecological spatial information of different ecosystem components into an overall map of marine biological value, highlighting hotspots of ecological significance. This study also evaluated the extent to which high biological value was contained in the current and projected Natura 2000 sites in Portugal.

By integrating the information of marine connectivity pathways, with a broad biological assessment of the Portuguese coast, this thesis seeks to build upon existing MPA assessment criterion and take a step forward in making it practical and applicable to the Portuguese setting.

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**Sleutelwoorden:**

Ecologische coherentie, beschermde zeegebieden, netwerken, connectiviteit, representativiteit, natuurlijke merkers, drones, biofysische modellering, biologische waardebeoordeling van zeegebieden

**abstract**

De internationale vraag naar een succesvolle ecosysteem-gebaseerde aanpak om de impact van menselijke activiteiten op de gezondheid van de oceanen te vermijden, te reduceren of te compenseren, neemt toe in populariteit. Belangrijke Europese maritieme beleidsinstanties en andere internationale agentschappen benadrukken dat het oprichten van ecologisch coherente netwerken van beschermde zeegebieden noodzakelijk is om het mariene milieu doeltreffend te beschermen.

Echter, het bereiken van ecologische coherentie is een complexe taak gezien het ruime concept en het ontbreken van eenduidige operationele definities. Het vaststellen van ecologische coherentie vereist dus verschillende benaderingen. Op die manier kan niet alleen de locatie, de grootte en afstand van reeds geselecteerde gebieden voor bescherming bepaald worden, maar ook nieuwe locaties die het netwerkeffect versterken. Het doel van deze thesis is het ontwikkelen en toepassen van verschillende methodes als hulpmiddel om de ecologische samenhang van beschermde zeegebieden te promoten, en dit met focus op continentaal Portugal.

Drie complementaire methodes werden toegepast om de connectiviteit via larvale dispersie in te schatten langs de Portugese westkust en dit met de mossel *Mytilus galloprovincialis* als modelorganisme. De eerste studie beschrijft het gebruik van natuurlijk voorkomende geochemische tracers om de oorsprong van de mossels te achterhalen en documenteert dus de demografische connectiviteitspatronen (verspreidingsroutes en afstanden). De focus lag op de beschermde zeegebieden van Arrábida en Berlengas, en benadrukte het belang van 'source-and-sink' populaties en zelf-recruterende gebieden. In een tweede studie werd de oppervlakte van mosselbedden gekwantificeerd zodat de connectiviteitsschattingen konden toegepast worden op modellen van larvale dispersie op grotere schaal. Het gebruik van beeldmateriaal met hoge resolutie verzameld door drones op lage hoogte, 3D-oppervlakte modellen en veldobservaties bleek een efficiënte methode om intertidale ecosystemen te bestuderen op een ecologisch relevante schaal. De combinatie van empirische connectiviteitsschattingen en demografische en biologische parameters (oppervlakte van mosselbedden, gedrag van larves, mortaliteit, reproductieve output en timing) laat de validatie toe van een grootschalig numeriek, biofysisch model. Het effect van de omgevingsvariabelen (zoals substratum complexiteit en blootstelling aan golven) op de mosseldichtheid en -grootte werd ook onderzocht. Maximum mosseldichtheid kwam voor bij intermediaire waarden van golfblootstelling in de winter en kleinere mosselen domineerden bij hoge waarden van blootstelling aan golven.



**abstract  
(cont.)**

Dit model combineert oceanografische en demografische informatie met larvale biologie, en werd gebruikt om de dispersie van *M. galloprovincialis* larven langs de West-Iberische kust te simuleren. Het biofysisch model hield rekening met de onzekerheid van de geochemische methode in het toekennen van de 'source' populaties en resulteerde in ongeziene overeenkomsten tussen de onafhankelijke connectiviteitsschattingen op kleine schaal. Vanaf nu kan dit verfijnde model gebruikt worden om de dynamieken van metapopulaties te onderzoeken en kan het dus bijdragen aan weldoordachte strategieën met betrekking tot het oprichten van beschermde zeegebieden

Tot slot werd een kwantitatief-gebaseerde ecologische aanpak gebruikt om de biodiversiteit te bepalen langs het Portugees continentaal plat. Het protocol voor mariene biologische waardebeoordeling combineerde bestaande biologische en ecologische informatie van verschillende subzones in een kaart die de biologische waardebeoordeling weergeeft, en hotspots van ecologisch belang identificeert. Deze studie evalueerde ook de mate waarin grote biologische waarde vervat zit in de huidige en toekomstige Natura 2000 gebieden in Portugal.

Door het integreren van informatie over mariene connectiviteitsroutes, met breed biologisch onderzoek langs de Portugese kust, bouwt deze thesis verder op het bestaande criterium voor de selectie van beschermde gebieden, en maakt het praktisch en toepasbaar voor Portugal.



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## Thesis Overview

Assessing ecological coherence of networks of Marine Protected Areas (MPAs) is a contemporary issue. Major European and other international agencies advocate that, in order to effectively protect the marine environment, a network of MPAs needs to be ecologically coherent. To achieve this goal, four main factors need to be taken into consideration: connectivity, adequacy, representativity, and replication. However, given the magnitude of the concept and the lack of clear operational definitions, building a coherent network of MPAs is a challenge. The aim of this thesis is not to provide an absolute assessment of “coherency” within the network of MPAs in Portugal, but rather to use and develop different tools to assist, support and help to promote this complex but crucial task.

The criteria of connectivity, adequacy, representativity, and replication ultimately reflect ideal MPA size, spacing, shape and the distribution and ecology of species and habitats at different scales. These concepts are reviewed in **Chapter I**, together with an overview of threshold levels used to assist in the guidance and measurement of coherence. This chapter highlighted the international pressure and the current constraints to set up coherent networks of MPAs so as to protect the structures and functions of marine systems, and promote economic and social benefits in an integrative manner. Also, it reflects on the current Portuguese situation in terms of marine protected areas’ implementation and management and the conservation strategies underlying the National Strategy for the Seas (ENM 2013-2020) and the Portuguese Natura 2000 sites. Chapters II to IV concentrate on tools to investigate population connectivity among MPAs, one of the overarching performance criteria of networks of MPAs. Three different but complementary methods were applied using the mussel *Mytilus galloprovincialis* as a model species to estimate larval dispersal along the Portuguese west coast. Chapter V, in turn, focuses on the intrinsic value incorporated in biodiversity per se, to detect hotspots of high biological value in the study area.

**Chapter II** describes the use of natural geochemical tags (of larval and recruit shells) to trace back natal origins of mussels and describe the connectivity patterns among the Arrábida and Berlengas Marine Protected Areas (MPAs) in the central Portuguese west coast. This chapter describes direct measures of demographic connectivity (dispersal trajectories and distances) for an important ecosystem engineer in rocky shores and emphasizes the significance of source and sink populations and self-recruitment within MPAs. Our results suggest that

protected sites should be placed within around 50 km from each other to maximize benefits for mytilid marine larvae with potential large-scale dispersal among rocky intertidal areas.

#### Research Questions:

1. Are geochemical signatures in larval shells adequate to distinguish natal sources of mussel recruits (spatial scale of geochemical variability)?
2. What are the main dispersal trajectories and distances and how much self-recruitment occurs during the study period?
3. What is the degree of connectivity between the two Portuguese MPAs and among these and the remaining coast?
4. Are the results consistent with simultaneous environmental and oceanographic data?

**Chapter III** resulted from the need to quantify mussel bed coverage in order to apply the connectivity estimates into broader scale models of larval dispersal. This way, this chapter describes the use of low altitude and high-resolution drone imagery, 3D surface models and ground based observations as a viable and powerful tool for surveying intertidal ecosystems. Aerial images were mosaicked and georeferenced and a 3D photogrammetric model was reconstructed with a ground resolution of less than 1cm/pixel. Mussel coverage, density and mean size was then calculated for each location and modelled along the central Portuguese west coast as a function of a wave exposure index.

#### Research Questions:

1. Are low altitude-high resolution aerial images useful in investigating fine-scale rocky intertidal topography and mussel coverage in rocky intertidal shores?
2. Does combining aerial images with 3D photogrammetric models and ground-based quadrat sampling provide realistic measurements of mussel bed coverage, density and size?
3. Are wave exposure and terrain roughness good explanatory variables of mussel coverage and size?

**Chapter IV** puts together the results of Chapters II and III by comparing the empirical connectivity matrices obtained with geochemical tags with predicted connectivity matrices produced by a numerical biophysical model. This model combined oceanographic and demographic information with larval biology, which was used to simulate dispersal of *M. galloprovincialis* larvae along the study area. In addition to predicting larval dispersal, this



chapter uses the biophysical model to simulate and account for uncertainty of the geochemical method in the assignment of recruits to putative source populations, thereby improving the cross-validation of two independent estimates of marine population connectivity.

Research Questions:

1. What is the current situation on scientific studies dealing with multiple methodological approaches to increase confidence in estimates of population connectivity?
2. Does accounting for uncertainties in larval origins derived from elemental chemistry improve concordance with predictions from a biophysical model of dispersal?

**Chapter V** explores a quantitative-based ecological approach which combines and spatially evaluates data for a wide taxonomic range of ecosystem components (seabirds, demersal fish and invertebrates, macrobenthos, marine mammals and sea turtles) at the scale of tens of kilometres. The Marine Biological Valuation (MBV) protocol was applied along the continental Portuguese continental shelf to create maps describing patterns of biological value and biodiversity hotspot areas. This study also considered the extent to which high biological value was contained in current Natura 2000 Special Protection Areas and projected Sites of Community Importance in Portugal.

Research Questions:

1. Which databases are available on the distribution and abundance of different ecosystem components (seabirds, demersal fish, macrobenthos, marine mammals and sea turtles) at the scale of tens of kilometres along the continental Portuguese shelf?
2. Does the data have enough spatial and temporal resolution to apply the marine biological valuation protocol?
3. What is the spatial overlap of the high valuable areas (hotspots) with current and prospected marine conservation areas (Natura 2000 network)?
4. What is the significance of the results in the context of the Portuguese marine spatial planning and conservation strategies?

Finally, **Chapter VI** builds upon the overall findings and integrates and discusses the results within the context of the assessment of MPA networks at the national scale. It also briefly provides some general guidelines for future work, in order to use the best available scientific information for the design and assessment of the network. By integrating the information of marine connectivity pathways with a broad biological assessment of the Portuguese coast, this

thesis hopes to assist in the ongoing process of MPA design and assessment and take a step forward in the guidance of conservation management applicable to the Portuguese setting.

The results of this thesis have been partially presented at various national and international conferences and published (or submitted) to peer-reviewed journals, as follows:

Peer reviewed publications:

Gomes I, Peteiro LG, Bueno J, Albuquerque R, Nolasco R, Dubert J, Queiroga H, 2018. What's a picture really worth? On the use of drone aerial imagery to estimate intertidal rocky shore demographic parameters (to be submitted to *Remote Sensing of Environment*).

Gomes I, Pérez-Jorge S, Peteiro L, Andrade J, Bueno-Pardo J, Quintino V, Rodrigues AM, Azevedo M, Vanreusel A, Queiroga H, Deneudt K, 2017. Marine biological value along the Portuguese continental shelf; insights into current conservation and management tools. (under review) *Ecological Indicators*.

Nolasco R, Gomes I, Peteiro L, Albuquerque R, Luna T, Dubert J, Swearer SE, Queiroga H, 2018. Independent estimates of marine population connectivity are more concordant when accounting for uncertainties in larval origins. *Scientific Reports*. 8 (1) pp: 2641 [doi:10.1038/s41598-018-19833-w](https://doi.org/10.1038/s41598-018-19833-w)

Gomes I, Peteiro LG, Albuquerque R, Nolasco R, Dubert J, Swearer SE, Queiroga H, 2016. Wandering mussels: using natural tags to identify connectivity patterns among Marine Protected Areas. *Marine Ecology Progress Series*. 552, 159–176 <https://doi.org/10.3354/meps11753>

Oral communications:

Queiroga H, Gomes I. 2018. Valor biológico e conectividade populacional: ferramentas complementares para designar redes de áreas marinhas protegidas. Primeiro Congresso de Biologia Marinha dos Países de Língua Portuguesa. Faro, Portugal. (oral presentation)

Gomes I, Peteiro L, Albuquerque R, Bueno J, Nolasco R, Dubert J, Swearer S, Queiroga H, 2017. Connectivity and Demographic insights of *Mytilus galloprovincialis* in the Portuguese west coast. 2nd International Symposium on the Advances in Marine Mussel Research. Sète, France. (oral presentation)

Andrade J, Albuquerque R, Azevedo M, Pardo JB, Deneudt K, Dubert J, Gomes I, Nolasco R, Perez S, Peteiro L, Quintino V, Swearer SE, Vanreusel A, Queiroga H 2017. Biological

value and population connectivity assessments: complementary tools to designate networks of MPAs. III Internacional Workshop LIFE+ MarPro. Ilhavo, Portugal. (oral presentation)

Gomes I, Peteiro LG, Bueno J, Albuquerque R, Nolasco R, Dubert J, Queiroga H, 2016. What's a picture really worth? On the use of drone aerial imagery to monitor intertidal rocky shores. MARES Conference II, Marine ecosystem Health and Conservation, Olhão, Portugal.

Nolasco R, Gomes I, Peteiro L, Albuquerque R, Dubert J, Swearer SE, Queiroga H, 2016. Accounting for uncertainty improves cross-validation of independent estimates of larval dispersal and population connectivity in a spatially structured marine metapopulation. Second Meeting of the International Marine Connectivity Network - iMarCo, St. Andrews, Scotland. (oral presentation)

Gomes I, Pérez-Jorge S, Peteiro L, Andrade J, Bueno-Pardo J, Quintino V, Rodrigues AM, Azevedo M, Vanreusel A, Queiroga H, Deneudt K, 2016. On the ecological coherence of Marine Protected Areas. Marine Biology Section lab Symposium. Ghent University, Belgium.

Gomes I, Peteiro LG, Albuquerque R, Bueno J, Pimentel J, Nolasco R, Dubert J, Swearer S, Queiroga H, 2015. Identificação de padrões de conectividade populacional entre áreas marinhas protegidas. I Jornadas do conhecimento da Reserva da Biosfera das Berlengas (UNESCO) Escola Superior de Turismo e Tecnologia do Mar. Peniche, Portugal.

Gomes I, Peteiro LG, Bueno J, Albuquerque R, Queiroga H, 2015. Quanto vale uma fotografia? Sobre o uso de drones na monitorização do intermareal rochoso. IV Encontro Nacional de Pós-graduação em Ciências Biológicas. Universidade de Aveiro, Portugal.

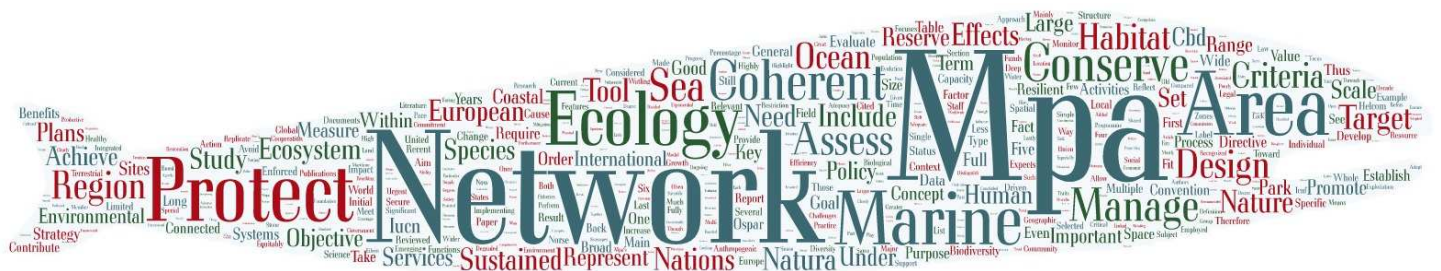
Gomes I, Peteiro LG, Albuquerque R, Swearer SE, Queiroga H, 2014. Wandering Mussels: using natural tags to identify connectivity matrices amongst Marine Protected Areas. MARES Conference I, Marine ecosystem Health and Conservation, Olhão, Portugal. (oral presentation)

Gomes I, Peteiro LG, Albuquerque R, Swearer SE, Queiroga H, 2014. Wandering Mussels: using natural tags to identify connectivity matrices amongst Marine Protected Areas. International Council for the Exploration of the Sea Annual Science Conference 2014 – Sustainability in a changing ocean. La Coruña, Spain. (oral presentation)

Gomes I, Peteiro LG, Albuquerque R, Swearer SE, Queiroga H, 2014. Using trace element content in mussel larval shells to identify natal sources. Estuarine and Coastal Sciences Association Conference ECSA. Sesimbra, Portugal. (oral presentation)

Distinctions and awards:

- Mussels used to map habitat connectivity of Natura 2000 marine sites in Portugal. 2016. Science for Environment Policy information service. Published by the European Commission Directorate-General Environment, Issue 475.
- Best oral presentation, under the theme Biodiversity Effects. 2016. MARES Conference II, Marine ecosystem Health and Conservation, Olhão, Portugal.
- Sesimbra Scientific Award and science outreach book publication. Gomes I, Peteiro LG, Albuquerque R, Nolasco R, Dubert J, Swearer SE, Queiroga H, 2016. A viagem do mexilhão da Arrábida, *ou como para viajar, basta existir*. 2015. Câmara Municipal Sesimbra.
- Best PhD student oral presentation. 2015. Encontro Nacional de Pós-graduação em Ciências Biológicas. Universidade de Aveiro, Portugal.



## Chapter I

### Introduction

Overview of the current status of the criteria used to designate and assess networks of Marine Protected Areas (MPAs).

## Introduction

Coherent networks of MPAs in Europe have been largely driven by the Natura 2000 process, by regional seas conventions, and backed up by national legislations. Rather than exhaustingly define all criteria and target levels used in many contexts, and already reviewed in several policy documents, technical reports and publications (see Ardron 2008, HELCOM 2010, Olsen et al. 2013, Wolters et al. 2015), this section focuses on key guiding principles and criteria supporting the design and assessment of the multi-faceted model for ecologically coherent networks of MPAs.

### 1.1 The evolution of MPAs as conservation management tools

A broad spectrum of atmospheric, land and ocean anthropogenic based activities, together with the exponential growth of the human population and coastal relocation, is causing complex modifications in the physical structure (e.g. Watling and Norse 1998), chemistry (e.g. Boesch et al. 2001) and ecology (Lubchenco 1995, Botsford et al. 1997, Vitousek et al. 1997, Hutchings 2000, Jackson et al. 2001) of the oceans, and consequently in the ecosystem services they provide (Worm et al. 2006).

The marine environment, once considered a resilient and inexhaustible ecosystem, is now facing cumulative anthropogenic stressors (Halpern et al. 2007) that are changing the structure, dynamics and functioning of ecosystem and its ability to supply goods and services to society (Cardinale et al. 2012). Furthermore, research suggests that the projected pace of human population growth will contribute substantially to environmental degradation (Crist et al. 2017). As a result, there is an urgent need in achieving high standards of sustainable human development while protecting biodiversity. This notion is an important foundation stone for several and contemporary regional and global commitments and is included in the 2030 United Nations Agenda for Sustainable Development Goals (SDGs): *“To sustainably manage and protect marine and coastal ecosystems to avoid significant adverse impacts, including by strengthening their resilience, and take action for their restoration in order to achieve healthy and productive oceans”*. In fact, the United Nations just announced the Decade of Ocean Science for Sustainable Development (2021-2030), an initiative to boost international cooperation in ocean sciences.

In both terrestrial and marine systems, the creation of protected areas is a cornerstone tool to promote conservation and manage human activities and sustainable resource exploitation (Gaines et al. 2010). Marine Protected Areas (MPAs) are defined by the International Union for Conservation of Nature (IUCN) as *“a clearly defined geographical space, recognized, dedicated and managed, through legal or other effective means, to achieve long-term conservation of nature with associated ecosystem services and cultural values”*. Therefore, MPAs serve as spatial administrative tools which encompass a variety of conservation and management methods, employed in a wide range of habitats including the open ocean, deep sea, coastal areas, inter-tidal zones, estuaries and lakes. However, the employment of the label “Marine Protected Area” diverges amongst international agencies and national authorities, and comprises a large variety of zoning and management schemes, from multiple use to fully no-take no-entry zones. This variability provides little information on the whole-ecosystem conservation and might prevent a correct evaluation of the existing types of MPAs and their efficiency (Horta e Costa et al. 2016). In fact, when Costello and Ballantine (2015) analysed the 2013 World Database on Protected Areas (United Nations Environmental Programme), they found out that protection is generally weak: 94% of designated MPAs allow fishing and less than 1% of the ocean is a no-take reserve.

There is an extensive and growing body of scientific evidence regarding marine protected areas ecological, social and economic benefits. Yet, MPAs have also been criticized to cause social and economic constraints for local communities. This way, there is a consensus that a simple label of MPAs cannot guarantee long term conservation objectives, especially if they are selected under ineffective design (Cox et al. 2017) and if there are large shortfalls in our capacity to manage, monitor and finance those areas (Bennett and Dearden 2014).

In general, MPAs' benefits and/or challenges (Table 1) largely depend on MPA purpose, location, design, management approaches and level of protection, funding and restrictions on human uses. Edgar et al. (2014) investigated 87 MPAs worldwide and documented that conservation benefits increase exponentially with the accumulation of five key features: old (>10 years), large (>100 km<sup>2</sup>), no take areas, well enforced management plans and isolated by deep water or sand. Yet, a recent study by Gill et al. (2017), reporting on 218 MPAs worldwide, concluded that staff and budget capacity were the strongest predictors of conservation impact; MPAs with adequate staff capacity had ecological effects 2.9 times greater than MPAs with inadequate capacity.

Table 1.1 Major MPAs' benefits and/or challenges and examples cited in the literature.

Benefits		Challenges	
Description	Examples	Description	Examples
Protecting biodiversity and increasing productivity	Halpern and Warner, 2002 Mumby et al. 2007 Russ and Alcala, 1996 Lester et al. 2009	Lack of proper resources, planning, or enforcement mechanisms	Bruner et al. 2004 Byers and Noonburg, 2007 Guidetti et al. 2008 McCay and Jones, 2011 McClanahan et al. 2006 Rife et al. 2013 Rojas-Bracho et al. 2006
Providing Ecosystem Services	Potts et al. 2014 Leenhardt et al. 2015 Xu et al. 2017		
Increasing ecologic resilience	Babcock et al. 2010 White et al. 2012		
Increasing recreational and tourism opportunities	Taylor and Buckenham, 2003 Weiant and Aswani, 2006 Hayes et al. 2015	Tourism	Harriott et al. 1997
Enhancing of fisheries	Gell and Roberts, 2003 Harrison et al. 2012 Moland et al. 2013	Negative impacts on fisheries	Caveen et al. 2014
Cost	Balmford et al. 2003	Costs and/or legal context	Lowry et al. 2009 McCrea-Strub et al. 2011
Supporting health, social or cultural values	Aswani and Furusawa, 2007 Cinner et al. 2005 Gjertsen, 2005 Pollnac et al. 2010	Social constraints	Bennett and Dearden, 2014 Himes, 2007 Mascia et al. 2010 West et al. 2006)
Protecting specific habitats, species or functional groups	Fish, Russ and Alcala, 1996 Megafauna, Hooker and Gerber, 2004 Penguins, Pichegru et al. 2010 Sharks, Knip et al. 2012 Dolphins, Pérez-Jorge et al. 2015 Seabirds, Maxwell et al. 2016 Coral reefs, McClanahan et al. 2007 Habitats, Frascchetti et al. 2013	Disadvantages of very large marine protected areas and/or rush to achieve percentage targets	Agardy et al. 2011 De Santo, 2013 Devillers et al. 2015 Jones and De Santo, 2016 Sheppard et al. 2012 Singleton and Roberts, 2014 Wood, 2011

Despite some ongoing debate on conservation needs and priorities, MPAs have materialized as a mainstream management tool for promoting long-term conservation and sustainable use of marine resources (Halpern and Warner, 2002), and symbolize a key task for different EU coastal, marine and biodiversity policies. Still, the progress towards protecting coastal and marine areas has been much slower than their terrestrial equivalent (Fig. 1.1, Watson et al.



2014). Even though only a small fraction of the ocean is protected, a considerable progress has been made in the last decade, with an increase from less than 0.1% to 1.6% of marine areas protected (Lubchenco and Grorud-Colvert 2015). This rise is expected to continue in the next years, with the emerging trend for establishing and implementing remote large-scale marine protected areas within and beyond national jurisdiction (Leenhardt et al. 2015)

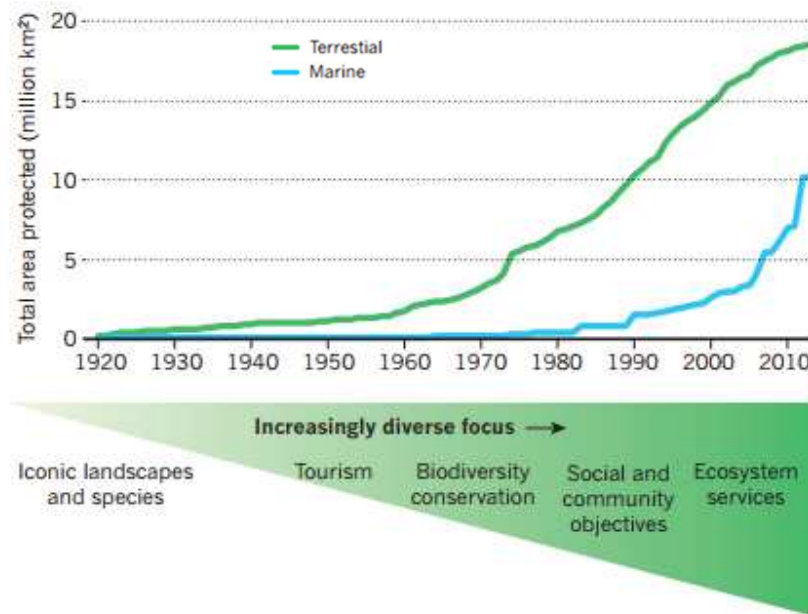


Figure 1.1 Growth of the modern terrestrial and marine protected area estate (World Database on Protected Areas) and their relation with a series of the evolution of concepts and goals over the past 150 years (image taken from Watson et al. 2014).

While the broad objective of the initial movement for protected areas was to conserve iconic and vulnerable seascapes and species, their purpose has changed over the past century towards the emergence of a much more systematic and ecosystem-based approach. A shift in protected areas philosophy promotes the protection of the structures and functions of marine systems, the provision of crucial ecosystem services (food security, human health, carbon storage), contribution to national and local economies (replenish fisheries, tourism revenues), and expects them to play a key part in the resilience and mitigation of climate change (Watson et al. 2014, Gormley et al. 2015).

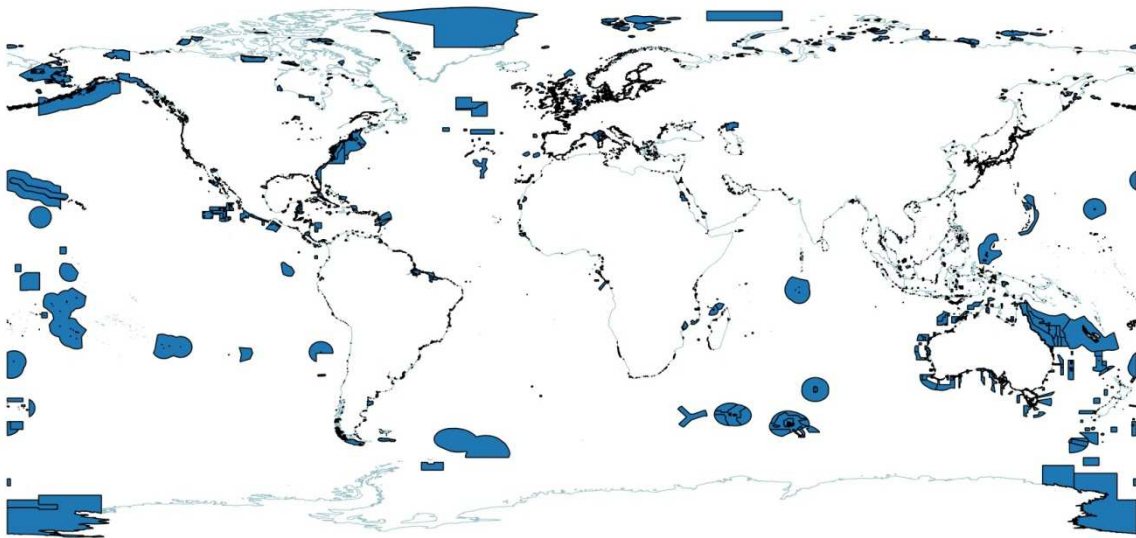


Figure 1.2 Map showing current worldwide MPAs (blue polygons and dark coastal contour). This database is updated monthly and managed by the United Nations Environment World Conservation Monitoring Centre (UNEP-WCMC) with support from IUCN and its World Commission on Protected Areas (WCPA). At present, the database reports 15 609 designated, inscribed, and/or established MPAs, includes 25,245,207 km<sup>2</sup> of marine areas and covers 6.97% of the total ocean (<https://protectedplanet.net/marine>).

In general, MPAs' benefits and/or challenges largely depend on MPA purpose, location, design, management approaches and level of protection, funding and restrictions on human uses. Currently, MPAs should no longer be established on an individual ad hoc basis, but scale up to a network approach, considering the protection of the structures and functions of marine systems, in an integrative manner.

## 1.2 From individual MPAs to Networks of MPAs

At an international level, the fact that MPAs should work in synergy with each other has been comprehensively outlined by the International Convention on Biological Diversity (CBD) Aichi Biodiversity Target 11. This percentage-based target for marine conservation, signed by over 150 government leaders, states that “by 2020 at least 10% of coastal and marine areas, especially *areas of particular importance for biodiversity and ecosystem services, would be conserved through effectively and equitably managed, ecologically representative and well-connected systems of protected areas and other effective area-based conservation measures that are integrated into the wider seascapes.*” This way, simply reaching large percentage area

coverage of MPAs is not sufficient to meet the requirement of ‘ecologically representative and well-connected systems’ defined by the CBD.

A more ambitious goal was set by the UCN World Parks Congress 2014 in Sydney, with a recommendation to *“urgently increase the ocean area that is effectively and equitably managed in ecologically representative and well-connected systems of MPAs or other effective conservation measures. This network should target protection of both biodiversity and ecosystem services and should include at least 30% of each marine habitat. The ultimate aim is to create a fully sustainable ocean, at least 30% of which has no extractive activities.”*

The term ‘network of MPAs’ is therefore frequently used in environmental policies and guidelines, but it is still poorly defined. In this sense, it is important to distinguish a network of MPAs from a set of MPAs (Roff 2005). While a set of MPAs is any group of protected areas within a defined geographic region, a network of MPAs is a “a collection of individual MPAs operating cooperatively and synergistically, at various spatial scales, and with a range of protection levels designed to meet objectives that a single reserve cannot achieve” (IUCN-WCPA 2008). It is then expected that the network increases the ecological, social and economic benefits when compared to the individual performance of its constituent MPAs. Planning for the networks requires careful considerations of their purpose for implementation, design, size, spacing and level of protection of the individual MPAs. Several studies have already highlighted the ecological benefits of networks of MPAs (Sala et al. 2002, Planes et al. 2009, Christie et al. 2010, Grorud-Colvert et al. 2014).

In European waters, MPA networks have largely been driven by the European Union (EU) Natura 2000 network, an important environmental pillar of the wider EU Integrated Maritime Policy. This ongoing process aims to develop a *coherent network of special areas of conservation to protect threatened species and vulnerable habitats*. The marine area included in the network doubled in the last five years and is currently comprised of more than 3140 marine sites, covering around 7% of EU seas (Natura barometer October 2017).

Additionally, the Marine Strategy Framework Directive (MSFD) stipulates that Member States need to include into their programmes *“spatial protection measures, contributing to coherent and representative networks of MPAs”*, so as to support a Good Environmental Status (GES) in the marine environment by 2020.

Moreover, the European Regional Seas Committees (RSC) promotes the establishment of a coherent network of MPAs to ensure the sustainable use, protection and conservation of marine biological diversity and its ecosystems (e.g. OSPAR 2008, HELCOM 2010).

At the national level, the Portuguese government recently adopted the National Strategy for the Seas (ENM 2013-2020) (Resolution of the Council of Ministers No. 12/2014 of 12 February. D.R. No. 30, Series I). It promotes *“the establishment of a network of marine protected areas, efficiently managed, coherent and adapted to the territory within the framework of the international commitments and the national strategy for nature conservation, in order to recover degraded ecosystems and promote its potential as a recruitment area, thus contributing to improve the efficiency of activities, in particular fisheries.* Importantly, it also refers that the *“the delimitation of new marine protected areas, as well as the management plans implementation, require the scientific recognition of the natural values and its impacts and pressures, thus contributing to consolidate the process of the Natura 2000 network to the marine environment.”*

In mainland Portugal, which is the focus of this thesis, the establishment and management of protected areas (at national and regional level) is a competency of the Institute for Nature Conservation and Forests (ICNF) in articulation with the Directorate-General of Natural Resources, Security and Maritime Services. The designation of areas classified under the Natura 2000 network, RAMSAR Convention, European Network of Biogenetic Reserves (Council of Europe) and the Biosphere Reserves (Man and Biosphere - UNESCO) – are also a responsibility of ICNF. There are different types of protected areas with different management objectives and regimes according to the protection category: natural monuments, natural reserves, natural parks, national parks, protected landscapes, classified sites, private protected areas and “marine parks or “marine reserves”.

Six national protected areas in mainland Portugal include a maritime area, hereafter called national marine protected areas. They cover up 479 km<sup>2</sup>, representing 0.03% of the Portuguese territorial waters with a combined no-take area barely covering 5 km<sup>2</sup>. Their sizes range from (2.2 km<sup>2</sup> to 253.4 km<sup>2</sup>) with an average of average 61.2 km<sup>2</sup>, and are separated from each other an average distance of 80 km (Abecasis et al. 2017). Regarding the Natura 2000 network, Portugal has already designated 7 marine Special Protection Areas (SPAs) under the Birds Directive covering an area of 6188 km<sup>2</sup>. As for the Habitats Directive (Sites of Community Importance, SCIs) Portugal already designated 4 costal SCI, and there is a formalized national proposal for the creation and expansion of 3 marine SCIs, which awaits the approval by competent national authorities. While SPAs are designated directly by Member States, SCIs are first proposed by Member States and, when approved by the European Commission, are designated as Special Areas of Conservation (SACs). Portuguese MPAs, designated Natura 2000 SPAs and SCIs and currently proposed SCIs in continental Portuguese shelf waters, are mapped in Fig. 1.3, 1.4 and described in table 1.2.

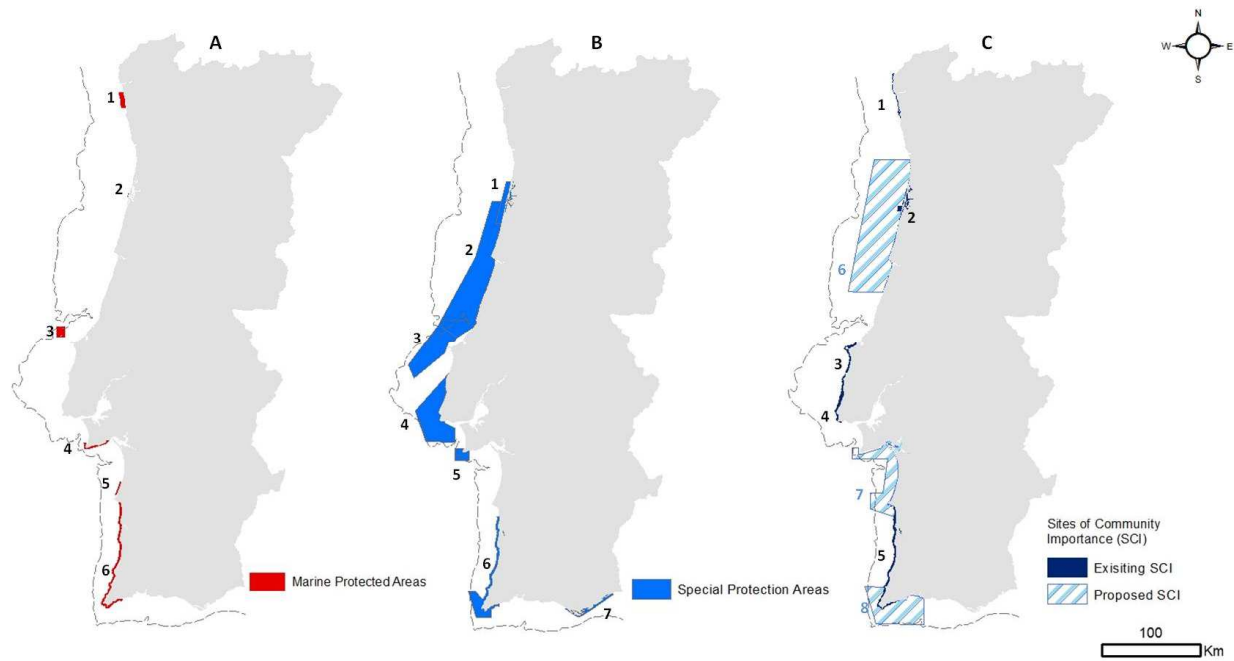


Figure 1.3 **A** - Current Portuguese MPAs **B** – Natura 2000 Special Protection Areas (SPAs) **C** - Existing and proposed Natura 2000 Sites of Community Importance. Banco Gorringe SCI is showed in figure 1.4. Dashed lines represent 200 m depth contours. Numbers in the maps are described in Table 2.

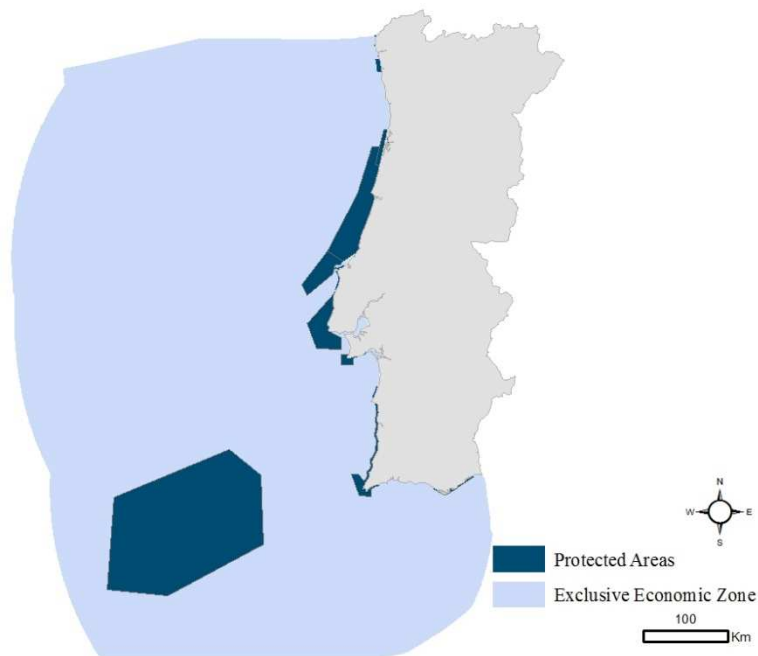


Figure 1.4 Overall Portuguese MPAs coverage in the Exclusive Economic Zone of mainland Portugal.

Table 1.2 Current Portuguese MPAs and Natura 2000 SPAs and SCIs (and proposed SCIs). Numbers represent areas in figures 1.3 and 1.4 and areas in brackets represent no-take areas.

	Classification Nº	Designation	Area (km <sup>2</sup> )	Creation (reclassification)	Management Plan (IUCN) category)	General objectives
National MPAs	Park 1	Litoral Norte	74.5	1987 (2005)	2008 (V)	Habitats, species and
	Reserve 2	Dunas de Sao Jacinto	2.6	1979 (2004)	2005 (IV)	cultural value
	Park 3	Berlengas	94.4	1981 (1999)	2008 (IV)	conservation
	Park 4	Arrábida	52.71 (4.32)	1971 (1976)	1980 (V)	Sustainable management of
	Reserve 5	Lagoas de Santo André Sancha	21.4	2000	2007 (IV)	economic activities
	Reserve 6	Sudoeste Alentejano e C. Vicentina	290 (0.63)	1988 (1995)	1995 (V)	and artisanal fisheries
Special Protection Areas (SPA)	1	Ria de Aveiro	264.0	1999		Maintenance/restoration of conservation
	2	Aveiro/Nazaré	2929.3	2015		status of birds species
	3	Ilhas Berlengas	1026.6	1999		listed in Annex A-I to
	4	Cabo Raso	1335.0	2015		Decree-Law No 140/99,
	5	Cabo Espichel	155.8	1999		and their habitats; and
	6	Costa Sudoeste	552.3	1999		migratory species with
	7	Ria Formosa	103.3	1999	under discussion	regular occurrence in the national territory.
Natura 2000	1	Litoral Norte	17.93	2004		Contribute to
	2	Ria de Aveiro	80.8	1999		biodiversity protection
	3	Peniche/Santa Cruz	57.99	2000		through the
	4	Sintra/Cascais	93.13	1997		conservation of
	5	Costa Sudoeste	202.5	1997		threatened natural
	6	Banco Gorringe	22887.82	2015		habitats and species listed in the Habitats Directive nº 92/43/CEE
Proposed expansion or creation of SCIs	6	Maceda/Praia da Vieira	5026			
	7	Costa de Setúbal	1233			
	8	Costa Sudoeste	1632			
TOTAL	Total area designated		30242 (4.95)			
	% of mainland EEZ protected		22% (0.0015%)			
	Additional area proposed		7891.0			

\*The total area of Portuguese continental Exclusive Economic Zone (EEZ) is 327,667 Km<sup>2</sup>.

\*\*According to the IUCN categorization of protected areas, **Category IV** (Habitat/Species Management) - protected areas aimed to protect particular species or habitats and management should reflect this priority. **Category V** (Protected Landscape/Seascape) - A protected area where the interaction of people and nature over time has produced an area of distinct character with significant, ecological, biological, cultural and scenic value: and where safeguarding the integrity of this interaction is vital to protecting and sustaining the area and its associated nature conservation and other values.

Networks of MPAs should operate cooperatively and synergistically to meet objectives that a single reserve cannot complete. In Europe, the process of creating networks of MPAs have been largely motivated by the CBD international target for marine protection, and driven by the Natura 2000 process, by Regional Seas Conventions, and backed up by national legislations.

### 1.3 The concept of ecological coherence for networks of MPAs

To make things more complicated, the expression network of MPAs in environmental policies is usually associated with the concept of ecological coherence, whose definition can be even more ambiguous. The term ‘ecological coherence’, lacks a theoretical foundation in ecological conservation science, but is thoroughly used in key European environmental policy legal documents (reviewed in Catchpole 2013) and rarely employed in peer reviewed environmental publications. Results from a search on ISI Web of Knowledge for the words “ecological coherence” or “ecologically coherent” appearing in the title or abstract fields resulted in 80 publications, with only 25 studies referring networks of MPAs (Fig. 1.5A). Interestingly, the first peer reviewed study relating ecological coherence and networks of MPAs only came out in 2008 (Fig. 1.5B). That is, almost 16 years after the mention in the Habitats Directive (92/43/EEC Article 3) for the need of “a coherent European Ecological Network”, and five years after the ministerial commitment to achieving ecological coherence within the OSPAR and HELCOM networks of MPAs (JMM 2003).

From the analysis, 20% of the studies on ISI Web of Knowledge fit in terrestrial ecology field and habitat restoration and almost half of the studies belonged to microbiology research field (48%), mostly concerning bacterial communities (where “ecological coherence of a taxon” means sharing general life strategies or traits that distinguish them from members of other taxa).

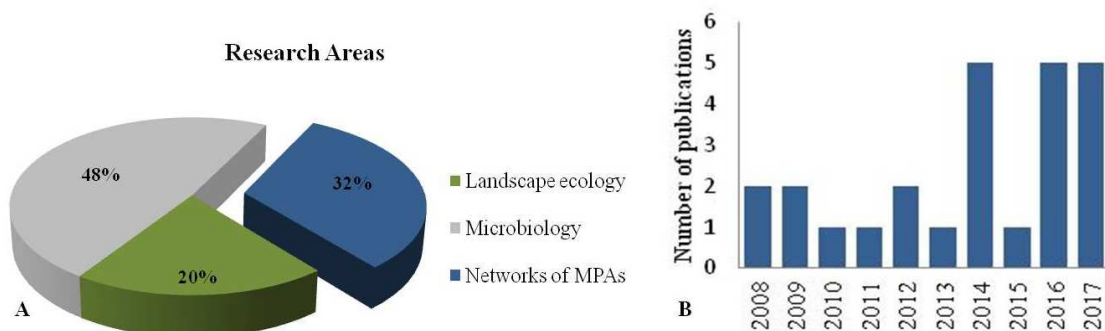


Figure 1.5 **A** - Results from a search on ISI Web of Knowledge for the words “ecological coherent” or “ecologically coherent” appearing in the title or abstract fields, separated by research areas. **B** - Results concerning the search described for the networks of MPAs category. The number of published articles per year is plotted. The graph starts with the first hit.

In Europe, the concept of ecological coherence is mostly linked with the Natura 2000 and regional seas conventions. In the Natura 2000 Habitats Directive (Council Directive 92/43/EEC;

EU, 1992), the term “ecological coherence” was made in the preamble to the Habitats Directive Article 3.1: “A coherent European ecological network of special areas of conservation shall be set up under the title Natura 2000”. In the context of the Natura Directive an ecologically coherent network should:

- i. represent the full range of variation in valued features;
- ii. replicate specific features at over a wide geographic area;
- iii. allow for dispersal, migration and genetic exchange of individuals between relevant sites, and include all critical areas for rare, highly threatened and endemic species;
- iv. be resilient to disturbance or damage caused by natural and anthropogenic factors.

A more comprehensive policy-based definition of ecological coherence comes from the OSPAR Convention (Ardron 2008): An ecologically coherent network of MPAs:

- i. Interacts with and supports the wider environment;
- ii. Maintains the processes, functions and structures of the intended protected features across their natural range;
- iii. Functions synergistically as a whole, such that the individual protected sites benefit from each other in order to achieve the other two objectives.
- iv. Be designed to be resilient to changing conditions.

In general, these concepts for ecological coherence are built upon many branches of ecological theories and supported by evidence-based research on: community ecology (e.g. Koelle and Vandermeer 2004, Leibold et al. 2004), ecological health (e.g. Lu and Li 2003), ecosystem integrity (e.g. De Leo and Levin 1997) and ecological resilience (e.g. Holling, 1996, Peterson 2000, Mumby et al. 2014, Scheffer et al. 2015). An integrated and consensual theory of “ecological coherence” is however, still missing.

The concept of ecological coherent in regards to networks of MPAs seems to be much more policy-driven rather than scientifically grounded. Coherent networks should deliver added ecological, economic, and social benefits through complementary outcomes of individual MPAs in fisheries management (Gell and Roberts 2003), biodiversity conservation (Almany et al. 2009) and thus assist in climate change adaptation and recovery after disturbance (Foley et al. 2010).



#### **1.4 Criteria used to assess the degree of ecological coherence of networks of MPAs**

Reserve network design, configuration (size, shape, spacing, and location) and management will determine the success and efficiency of achieving its initial goals. It is then necessary to find clear and measurable objectives to establish ecologically coherent networks of MPAs and demonstrate the 'added-value' of MPAs networks in relation to individual ones (Gorud-Colvert et al. 2014). For the purpose of this study, and in order to avoid multiple definitions and concepts, I have focused on four main criteria used to assess ecological coherence of networks of MPAs, recognized by the Convention on Biological Diversity (CBD 2008), the regional sea conventions (OSPAR 2007, HELCOM 2010,) and recommended by an independent study requested by the EU Directorate-General for the Environment (Wolters et al. 2015): representativity, replication, adequacy and connectivity.

The international guidance reports concerning network coherence show that there is a general concordance in these four criteria employed for the European marine regions. However, there are other relevant and comparable criteria cited in the literature. For example Catchpole (2013) underlined the importance of viability and resilience criteria; Lawton et al. (2010) gave more emphasis on anthropogenic factors, such as human accessibility and management and for Connor et al. (2002), representativity is called 'typicalness'. Moreover, CBD (2008) considered "Ecologically or biologically significant areas" as places providing important services to one or more species/ populations of an ecosystem or to the ecosystem as a whole, compared to other surrounding areas.

##### **1.4.1 Representativity**

As species diversity increases with habitat diversity, MPAs that include a variety of habitats and community types within well-connected networks, contribute to the persistence and resilience of ecosystems and ecological processes (Roberts et al. 2003). In this sense, scale is a key criterion to assess representative. While at a broader regional level, assessments can be made at a biogeographic regional scale defined by temperature and depth or incorporating major geomorphology (OSPAR 2013), at smaller scales, other units may constitute more ecologically meaningful surrogates for a representation. Examples include the use of marine landscapes (Golding et al. 2004), individual habitat types and species, such as rare habitats (Roberts et al. 2003) and areas of critical importance for different life stages of species (Roberts and Sargant 2008). However, the smaller the scale, the more refined and in-depth knowledge is required on the overall distributions of habitats and species.

### **1.4.2 Replication**

As stated by the CBD (2008): “All habitats within each region should be replicated and these should be spatially separate to safeguard against unexpected failures and collapse of populations”. Protecting a sufficient number of individuals of species, habitats and ecological processes in distant MPAs should promote ecosystem functions and spread over space the risks affecting MPAs units (Roberts et al. 2003) enhancing network resilience (IUCN WCPA 2008), providing new locations for colonization (Crowder et al. 2000) and/or stepping stones for dispersal of marine species (Cowen and Sponaugle, 2009). Local distribution, vulnerability, and pressure assessments at the habitat/species scale should determine the adequate number of replicates within the networks of MPAs.

### **1.4.3 Adequacy**

MPA network design should have an appropriate size and shape, as well as a satisfactory location and characteristics that minimise the impact of natural or anthropogenic threats and preserve the ecological integrity of the ecosystems (HELCOM 2010). Regarding the MPAs size, there has been a continuum debate concerning meta-population theory about the benefits of a single large or several small (SLOSS) protected areas in conserving biodiversity in a fragmented habitat over the long term (Wilcox and Murphy 1985). While large MPAs may support larger landscapes and populations, reducing edge effects (Aïramé et al. 2003), well inter-connected networks of smaller MPAs can support the persistence of populations at a greater extent (Zhou and Wang 2006) and maximize spill-over of propagules across the edges (Abesamis et al. 2006). As for MPAs shape, it should maximize the inclusion of targeted landscapes or habitats, and capture the gradient from onshore-offshore or habitat-habitat shifts of species of interest (IUCN-WCPA 2008). This way, adequacy of the network is mostly linked with its purpose, habitat extent and distribution, connectivity, population viability and anthropogenic threats.

### **1.4.4 Connectivity**

Connectivity expresses the extent to which populations are linked by the exchange of propagules (floating eggs, larvae, recruits, juveniles or adults), at the species' range (Palumbi 2003), and networks of MPAs should be composed of sites close enough to allow for sufficient exchange of adults and offspring. Consequently, optimal spacing of MPAs in a network is strongly influenced by the spatial scale of migration of the target species, and shaped by the physical environment, availability of suitable habitat, planktonic larval duration and behaviour,

extent of the source population (Gaines et al. 2010a, Krueck et al. 2017, Shanks et al. 2003) and MPA level of protection. Therefore, physical data such as habitat type and depth, oceanographic information (e.g. temperature, salinity, ocean currents) and biological data on larval dispersal distances for all target species, or groups of taxa with similar life cycles should be considered for a meaningful assessment of connectivity of networks of MPAs.

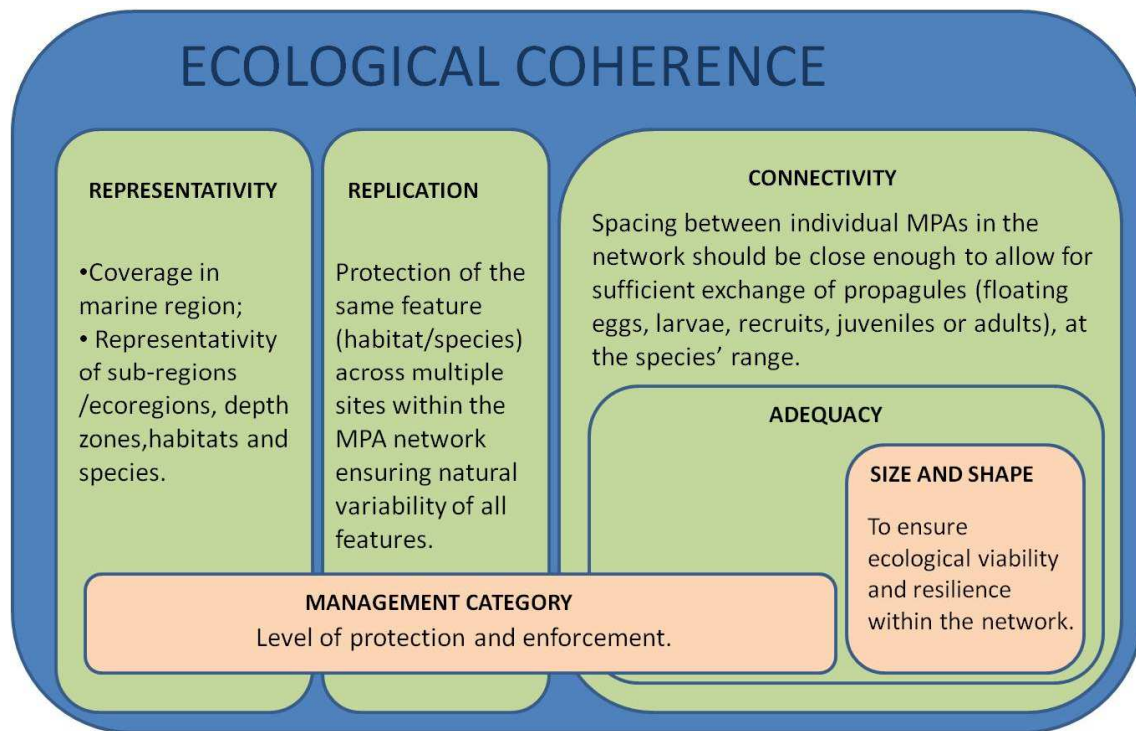


Figure 1.6 Overview of the main criteria, associated sub-criteria and concepts commonly used in the literature to define coherent networks of MPAs in the Europe (modified from Wolters et al. 2015).

Four consensual criteria are used to assess ecological coherence of networks of MPAs at a European level: representativity, replication, adequacy and connectivity. 'Coherence' is then regarded as an all-embracing principle, comprising the four main criteria.

### 1.5 Design and practical assessments of ecological coherence of networks of MPAs

In order to efficiently design networks of MPAs to achieve a set of specific objectives, there is no single one-size-fits-all ideal protocol. Different types of networks represent different goals and intended outcomes, and this should be considered when evaluating their performance (Grorud-Colvert et al. 2014). Networks of MPAs differ mainly in their spatial scale (from regional to local), their objectives and level of protection, ranging from broad conservation

approaches, to the protection of specific ecosystems and restriction of explicit activities (Table 1.3).

Table 1.3 Examples of networks of MPAs, goals and criteria guiding its implementation and monitoring.

Examples of MPA networks	General network goals	Design guiding criteria
Marine Natura 2000	The main objective of the network is to safeguard biodiversity in Europe; take all necessary measures to ensure a 'favorable conservation status' of species and habitat types of Community importance:	The Habitats Directive lists nine marine habitat types and 16 species for which marine site designation is required, whilst the Birds Directive lists a further 60 bird species whose conservation requires marine site protection.
OSPAR NE Atlantic MPA network	Make a significant contribution to the sustainable use, protection and conservation of marine biodiversity across the North-East Atlantic, including in Areas Beyond National Jurisdiction.	Representativity, replication, connectivity and adequacy (and resilience).
OSPAR High seas MPAs (areas beyond national jurisdiction)	Protection of vulnerable marine ecosystems (VMEs) from destructive bottom fishing.	Naturalness, practicality/feasibility, and biogeographic, ecological and scientific importance.
Great Barrier Reef (GBR)	Provide for the long-term protection and conservation of the environment of the GBR ecosystem; its outstanding universal value and its transmission in good condition to future generations and allowing ecologically sustainable use.	Biodiversity (habitats and species) and ecosystem health (physical, chemical and ecological processes and terrestrial habitats that support the Reef).
California coast MPA network	To protect the marine natural heritage, natural diversity and abundance of marine life, and the structure, function, and integrity of marine ecosystems; improving recreational, educational opportunities, and to manage these uses in a manner consistent with protecting biodiversity.	Representativity and replication of habitats in MPAs within a biogeographic region; size and spacing of MPAs to promote ecological connectivity
Kimbe Bay, Papua New Guinea MPA Network	To conserve marine biodiversity and natural resources (including a range of shallow water, deep water and island habitats), rare, vulnerable or threatened species and to address local marine resource management needs.	Biophysical design principles (representation, replication of habitat types and vulnerable areas, connectivity) and socio-economic design principles.
West Hawai'i MPA Network	To protect targeted species from aquarium fisheries; create fish replenishment areas, optimize coastal uses while meeting conservation targets.	Declare a minimum of 30 % of the West Hawai'i coastline as Fish Replenishment Areas, where aquarium fishing is prohibited.

Even though there is ample literature regarding suitable planning targets for the different criteria used in defining MPA ecological coherence, there are only few examples of comprehensive evaluations of the full network as a whole, considering all the established criteria. In the following paragraphs, I will focus on some of the most important efforts developed within the European context to evaluate ecological coherence within networks of MPAs; at a regional (NE Atlantic and Baltic Sea) and subregional level (Celtic seas) and describe the guidelines of an independent study commissioned by the EU to harmonise the methodology (Table 1.4 summarizes the major target and threshold levels currently used in European assessments, and includes examples at a global scale). Lastly, I reflect on the current status of the assessment of ecological coherence in Portuguese MPAs.

**1- OSPAR Commission** - The first comprehensive effort describing guidance on developing an ecologically coherent network came from the OSPAR Biodiversity Committee (Ardrón 2008). The Convention depicted ecological coherence as a holistic and relative concept, which should be measured as a probability that objectives were being met. In practice, the network performance results should fluctuate from ‘very unlikely to be ecologically coherent’ to ‘very likely to be ecologically coherent’. This way, three major complementary approaches were developed to assess eco-coherence (Ardrón 2008b), in an attempt to make use of available sources of information, balancing scientific rigor with political and administrative realities:

1. Expert knowledge self-assessments checklist and a scoring system;
2. Species-habitat tabular assessments against biogeographic region;
3. Three spatial assessments based on GIS data and single measure approaches, to use in data-limited situations, with thresholds levels for spatial distribution, representation, and threatened and/or declining species and habitats. The first test was on how well the network was spatially distributed and recommended maximum distances between MPAs of 250, 500 and 1000 in the nearshore, offshore and high seas respectively. The second test measured if the network covered at least 3% of most (seven of the ten) relevant Dinter biogeographic provinces. Finally, the third test evaluated that most (70%) of the OSPAR threatened and/or declining habitats and species were represented in the network, such that at least 5% (or at least three sites) of all areas within each OSPAR region in which they occur was protected.

In 2013, an independent assessment was tasked to apply and extend the methodology of the three initial tests agreed upon by OSPAR (Johnson et al. 2014). Briefly, this assessment concluded that, based on the agreed threshold distances, the OSPAR network of MPAs was not

spatially well-distributed, with the vast majority of MPAs being situated in coastal waters (0–75 m) and clustered around the North and Celtic sea. Regarding biogeographic representation and replication, the majority of biogeographic provinces surpassed the 3% threshold coverage. This assessment used data on Important Bird Areas as a proxy for assessing coverage of threatened/declining species and concluded that most of the offshore and high seas protection areas fell outside the OSPAR MPAs. Overall, this study concluded that in OSPAR Regions I (Arctic waters) and IV (Bay of Biscay and Iberian Coast) and large parts and depths of Region V (wider Atlantic) the network of MPAs was very unlikely to be ecologically coherent, while Regions II (Greater North Sea) and III (Celtic Sea) had a greater likelihood of being ecologically coherent. More recently, in the 2016 OSPAR status report (OSPAR 2016), the network was reevaluated and still not considered ecologically coherent. Nevertheless, it showed consistent improvements in the geographical distribution of MPAs in OSPAR Region IV (Bay of Biscay and The Iberian Coast), while the Arctic region remained with considerable gaps. An important and unrealistic assumption made by the OSPAR assessment is that all species and habitats are fully protected within all MPA boundaries, which is certainly not the case.

**2 – HELCOM** - In the recent Ecological Coherence Assessment of the Marine Protected Area network in the Baltic (HELCOM 2016), results showed a substantial overall protection of the Baltic Sea (aerial coverage of 12 %, the highest protection of all European marine regions). However, while representativity and replication were evaluated to be likely to have reached ecological coherence, adequacy was unlikely and connectivity very unlikely to fulfil the conservation goals of the MPA network. Therefore, the network was not considered ecologically coherent. However, when analysed together with marine Natura 2000 sites, the assessment reveals improved representativity, replication and connectivity.

**3 – Celtic Seas** - A very recent study by (Foster et al. 2017) represents the first assessment of the ecological coherence of a network of MPAs at a subregional level, analysing a network of 533 MPAs across the Celtic Seas and focusing on broadscale habitats. According to the adopted thresholds the Celtic Seas MPA network as a whole is not ecologically coherent; although it meets the desired CBD 10% spatial coverage for MPAs and all MSFD predominant habitat types are adequately represented and replicated within the network.

**4 - European Commission** - Recently, an independent study commissioned by the Directorate-General for the Environment (the European Commission department responsible for EU policy on the environment) proposed an assessment method to harmonise the methodology for the

evaluation of the coherence of the European networks of Marine Protected Areas (Wolters et al. 2015). The proposed method can be implemented and compared in different regions and scales across European seas, with heterogeneous ecological characteristics and data availability. With this method, all four main criteria (i.e. representativity, replication, connectivity and adequacy) must meet a minimum target level and the failure of a single one of the criteria results in a failure to reach ecological coherence. The assessment builds upon existing guidelines and involves a hierarchical approach depending mainly on the data availability. Basic assessments of ecological coherence can be achieved with GIS data on bathymetry, boundaries of the MPAs and territorial and exclusive economic zones, presence data of selected habitats, species and other features, and information on the protection level. A more detailed assessment can be accomplished using spatial data on habitat and species distribution and abundance, and main anthropogenic pressures. The process was tested in the central part of the Baltic Sea, and concluded that the network was unlikely to be ecologically coherent, as it failed to meet the targets for connectivity and representativity.

**4 – Portuguese MPAs assessment** - A systematic ecological coherence assessment including all protected areas for the Portuguese territorial waters is missing, as well as the definition of threshold levels to achieve it. Recently, Abecasis et al. (2017) used benthic habitat classification as a surrogate and baseline to assess MPA coverage and ecological coherence of the network of MPAs for the coastal waters of Portugal. Using Marxan and MinPatch solutions, the authors analyzed three conservation targets suggested in the MPA literature (10%, 30% and 50% protection) and concluded that, although most MPAs were above the minimum ecological size threshold (5 km<sup>2</sup>), the average distance between them was larger than the optimal distance of 10 to 20 km spacing suggested by (Shanks et al. 2003).

One of the key messages shared by all described assessment guidelines and protocols is that the broad assessment of the ecological coherence of networks of MPAs is highly compromised by data limitations on the distribution of species and habitats across broad geographic areas. Also, that there is a need of improved information on the management status and protection levels across MPAs, and a need for consistency and agreement of scientific based targets. These limitations are probably the underlining reason why no network has been considered coherent so far. Still, the growing availability of large scale knowledge and technology, mainly in species distribution, habitat and human impact mapping, is likely to promote and support the future progress in evaluating networks of MPAs.

Achieving ecological coherence relies on the network of MPA sites meeting a number of different criteria. Different target levels have been used in the design of coherent network of MPAs worldwide, depending mostly on data availability, quality and local requirements.

### **1.6 Challenges and limitations in assessing ecological coherence of networks of MPAs**

Reaching full ecological coherence implies having enough data to evaluate the interaction of different ecosystem components, structures and processes over wide spaces, to maintain a productive and healthy marine ecosystem. Hence, absolute “coherency” assessment remains on the theoretical realm.

Apart from the ecological criteria discussed above, there are many factors which can influence and restrain the coherence of the network. The first has to do with data quality, consistency and coverage. Regional assessments require a large amount and availability of data on substrate type, species distribution and ecological traits for a multiple range of functional groups, across multiple countries. Using broad scale data might misrepresent ecologically relevant processes at finer scales, and influence whether the “coherence” is accomplished at finer (national) or broader (European regions) scale.

An additional factor has to do with the fact that single MPAs and networks of MPAs are designated for different purposes, from a broad socio-ecological approach to specific species or habitats protection. This way, individual MPAs within the network may be designed for different purposes and managed under dissimilar strategies, and thus complicating the coherence assessment in multiple sites. A relevant example of these two constraints is the European Natura 2000 Marine Sites, which are selected and managed on a species/habitat specific level, with a strict selection of species and habitats. This has great implications for the assessment of the coherence of the network as a whole. So, the assessment of coherency requires the integration of those species/habitats listed in the respective habitats annexes, as well as the “full range of ecosystems, including the biotic and habitat diversity of those marine ecosystems’ (CBD 2008).

Given that all criteria to assess coherence is closely linked with the level of protection enforced inside the networks, some policy guidelines (e.g. Wolters et al. 2015) highlight the need to include “level of protection” when assessing ecological coherence of networks of MPAs. Also important is the need for cooperation in planning and assessing MPA designations at a regional and subregional scale, toward transboundary agreements to protect marine biodiversity in shared marine areas.



Therefore, even well thought, planned networks, theoretically fulfilling all ecological criteria, cannot grant conservation benefits if they are not sustained by adequate monitoring, management and effective enforcement on the ground (Pollnac et al. 2010). In fact, a recent study, Gill et al. (2017) concluded that whilst ecological inputs are important to determine MPA performance, investment in human and financial capacity are fundamental for effective protected area management. Furthermore, Kuempel et al. (2016) argued that limited funds should be disproportionately invested in enforcement rather than expansion of conservation areas.

Poor management, lack of incentives, insufficient protection levels and sectorial conflicts can transform good conservation intentions into a false sense of protection of marine ecosystems. The emergence of these “Paper parks” (Rife et al. 2013) can also be fuelled by political pressures to achieve international percentage-based targets, such as the CBD target to protect 10% of the ocean until 2020. Thus, and although percentage targets are a quantifiable simple metric chosen by the international community to communicate and promote conservation action, they are not a real measure of conservation success (Pressey et al. 2015). This is a delicate subject, given that area targets are easily quantifiable while management effectiveness is harder to measure. In this sense, the IUCN World Commission on Protected Areas approved in November 2017, a programme to enhance and distinguish effectively and equitably managed protected areas (IUCN Green List). Its mission is to ensure robust and impartial decisions based on expert judgment and verification of good governance, sound design, planning and effective management.

Lastly, even well managed ecologically coherent networks of protected areas will not fully sustain ecosystem protection and sustainable exploitation in marine ecosystems. Mitigating global threats also requires the adoption of management tools which secure protection outside MPA boundaries, alleviating pollution and promoting the sustainable development of human populations (Keller et al. 2009).

Many factors can influence and restrain the coherence of the network, mainly: poor data quality, the geographical scale of the assessment, the network levels of protection, poor monitoring, management and enforcement strategies, sectorial conflicts and lack of incentives and protection outside MPAs' boundaries.

Table 1.4 Summary of the major target and threshold levels used for the different criteria related to the assessment of coherent network of MPAs. The table focus at the European level, but shows examples of other metrics used worldwide and for different regions and ecosystems.

Main criteria	OSPAR 2008	HELCOM 2016	Celtic Seas MPA network	European Commission	Others
<b>Representativity</b>	Within each OSPAR biogeographic region, it is recommended that the OSPAR MPA network covers at least 3% of most (seven out of ten) relevant Dinter provinces.	<ul style="list-style-type: none"> <li>• &lt;20% coverage = inadequate protection;</li> <li>• 20-60% coverage = adequate protection of common habitats;</li> <li>• &gt;60% coverage = adequate protection of rare habitats.</li> </ul> ≥10% of the total Baltic Sea shall be protected, as well as each sub-basin and the coastal sea, outer coastal sea and open sea zones.	10% of coastal and marine areas; At least 3% of most of the relevant Dinter biogeographic provinces in the study area; Minimum patch size of 0.24 km <sup>2</sup> .	Subregions 10 % Depth zones 10 % Habitats 20 or 40 % Species 20 or 40 %	IUCN (2003) - At least 20-30% of each habitat should be included within the network. Barcelona Convention - In the Mediterranean, representativity of the most iconic or rare features (tentative target of 10%). Great Barrier Reef Marine Park (GBRMP) - minimum threshold of 20% of each bioregion to be protected within no-take zones.
<b>Replication</b>	Within each OSPAR biogeographic region, it is recommended that at least two MPAs for each EUNIS level 3 habitat be selected; for threatened and declining habitats and species, three replicate sites per biogeographic region is recommended.	A minimum of 3 replicates (of marker species, biotope complexes and benthic marine landscapes) within the HELCOM MPA network.	Replication thresholds: low (replication of habitat in 0- 2 MPAs), moderate (3- 5 MPAs), high replication (≥6 MPAs).	2 or 4 replicates of sites/features	IUCN-WCPA (2008)- at least three replicates per habitat type are included in the network; GBRMP- three to four replicates of no take zones for each bioregion (Fernandes et al. 2005); Irish Sea - habitats should be replicated in at least three protected areas spread throughout the Irish Sea (Roberts et al. 2003);
<b>Connectivity</b>	Recommendations for gaps between MPAs: Nearshore/Coastline: no gaps wider than 250 km and no more than 10 gaps • Offshore: no gaps greater than	50% of landscape patches should have ≥20 connections at the given dispersal distance; 50% of landscape patches representing habitats for	40 km buffer around MPAs	Between sites: 50 % of sites have ≥10 connections at 20 or 50 km distance (the distance is an	Shanks et al. (2003) - spacing of 10 to 20 km for species with typical pelagic larval durations Piekäinen and Korpinen (2007) – assessment of connectivity by calculating the proportion (%) of MPAs which are connected to X other MPAs within a selected distance (km).

	<p>a 500 km diameter circle (~200 000 km<sup>2</sup>) and no more than five gaps</p> <ul style="list-style-type: none"> <li>• High Seas: no gaps greater than a 1000 km square (1 000 000 km<sup>2</sup>) and no more than two gaps.</li> </ul>	<p>the species should have ≥20 connections at the given dispersal distance.</p>		<p>average for mobile species) Between features: 50 % of feature occurrences have ≥20 connections at 20 or 50 km distance.</p>	<p>IUCN-WCPA (2008) - spacing of 10 to 20 km up to 50 to 100 km between individual MPAs and recommends variable spacing, as opposed to even spacing. McLeod et al. (2009) - 15 to 20 km distance threshold between MPAs. Gaines et al. 2010 - 10 to 100 km distance between protected areas. Roberts et al. 2003 - sites in the network supporting similar habitats should be no more than 40 to 80 km apart.</p>
<b>Adequacy</b>	<p>Within each OSPAR biogeographic region, it is recommended that the OSPAR MPA network contain between 10-20% of each EUNIS level 3 habitat present and between 20% and 60% of the total extent of each OSPAR threatened and declining habitat. The sizes of network sites (for a given feature) should be distributed throughout, or exceed, the estimated range of sizes necessary to sustain a viable population or community for those species listed as threatened and/or declining.</p>	<p>80% of marine sites should be ≥ 30 km<sup>2</sup>.</p>	<p>20–30% of each habitat; Recommended size 10–100 km<sup>2</sup> Habitat patch size classes: 0–1 km<sup>2</sup> (sessile or very limited mobility species), 1–10 km<sup>2</sup> (low mobility), 10–50 km<sup>2</sup> (medium mobility), 50–100 km<sup>2</sup> (highly mobile), &gt;100 km<sup>2</sup> (very highly mobile).</p>	<p>Size: 75 % sites are &gt;20 km<sup>2</sup> or &gt;30 km<sup>2</sup> Protection level: 30 % or 40 % of sites are strictly protected</p>	<p>GBRMP - a minimum 20 km radius for no-take zones (Fernandes et al. 2005). Total MPA area that should be protected within a network: &gt;35% (Botsford et al. 2001); 40% (Sala et al. 2002); 30 - 50% (Aïramé et al. 2003b); 20 - 50% (Roberts et al. 2003); &lt;50% (Halpern and Warner, 2002); 20 - 30% (McLeod et al. 2009). New Zealand MPAs should have a minimum coastline length of 5-10 km, preferably 10-20 km, and should extend along the depth gradient from intertidal to deeper offshore waters, preferably to the 12 nautical mile limit. MPAs, with similar habitats should be placed within 50-100 km of each other (Thomas and Shears 2013). California preferred MPA size range 47 to 93 km<sup>2</sup> (Saarman et al. 2013)</p>
<b>Coherent?</b>	<b>No</b>	<b>No</b>	<b>No</b>	<b>No</b> (case study - central part of the Baltic Sea)	

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## Chapter II

### Wandering mussels: using natural tags to identify connectivity patterns among Marine Protected Areas

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**Abstract**

Connectivity in the marine environment is crucial for understanding the spatial structure of populations and for developing appropriate monitoring and management strategies. Here, we used the mussel *Mytilus galloprovincialis* as a model species to investigate connectivity patterns within the Berlengas and Arrábida Marine Protected Areas (MPAs) along the central Portuguese west coast. We generated an atlas of location-specific environmental markers based on the microchemistry of bivalve larval shells (using laser ablation inductively coupled plasma mass spectrometry). This atlas was then employed to trace back natal origins of newly settled mussels and generate connectivity matrices among populations. Our results reflected three distinctive chemical signatures in larval shells, corresponding to 3 regions: Estremadura, Cascais and Arrábida. Linear discriminant analyses allowed for a high reclassification success (average of 79.5% of jackknifed cross-validated cases correctly assigned) based on 8 of the 16 trace elements analyzed (B, P, Co, Cu, Zn, Ce, Pb and U). The population connectivity matrix identified different dispersal pathways for mussel larvae, in particular a predominantly northward dispersion pattern in July 2013. This pattern was consistent with simultaneous environmental physical data, which confirmed an extended period of wind reversal and upwelling relaxation. The Arrábida MPA was an important source population for the other two regions and showed high rates of self-recruitment but limited connectivity to the Berlengas MPA. These direct measures of demographic connectivity can be a powerful tool to inform policymakers on the conservation and management of ecological coherent networks of protected areas in coastal marine ecosystems.

**Keywords:** natal site atlas, *Mytilus*, elemental composition, LA-ICPMS, connectivity

## 2.1 Introduction

Measuring the spatial extent over which marine subpopulations are connected by larval dispersal is a fundamental issue in marine metapopulation studies (Pineda et al. 2007) and in defining the relevant spatial scales for area-based conservation measures (Gaines et al. 2010). Evidence from various fields such as physical oceanography, biophysical modelling, molecular genetics and the geochemistry of site-specific natural tags have been used to quantify connectivity at different spatial and temporal scales. Indeed, natural tags, such as the geochemical composition of calcified structures of marine organisms are increasingly being employed as a strategic tool in marine research. These naturally induced marks have been commonly used in paleo-environmental research in coral skeletons (Mitsuguchi et al. 1996), foraminiferal shells (Keul et al. 2013) and ostracod shells (Börner et al. 2013). More recently, they have been applied to determine natal signatures and dispersal patterns, using crustacean embryos and larvae (DiBacco and Levin 2000, Carson 2010), fish otoliths (e.g. Swearer et al. 1999), larval mollusk statoliths (Zacherl et al. 2005) and shells (e.g. Becker et al. 2007, Carson 2010). This method requires not only the existence of location-specific chemical signatures at the site of origin and the maintenance of these “natal tags” after settlement (Thorrold et al. 2007). Both physical and biological properties of the marine environment can influence the incorporation of such chemical signatures in biogenic carbonates at spatial scales over which they allow discrimination among natal sites (Campana 1999). Yet, the lack of a clear relationship between seawater chemistry and elemental composition of calcified structures (Campana and Thorrold 2001, Warner et al. 2005) might also reflect genetic (Chittaro et al. 2006) and/or maternal (Lloyd et al. 2008) effects on elemental signatures.

Assembling regional chemical reference maps of natal origins based on geographical differences in biogenic carbonate chemistry can be used as a tracking method (Becker et al. 2007). However, in order to successfully set up a suitable natal site atlas, it is crucial to consider not only the larval life history and potential dispersal scales, but also the local geology, anthropogenic pressures, and oceanography of the study region (Miller et al. 2013). This approach has already led to important progress in our understanding of metapopulation connectivity in coral reef ecosystems (Swearer et al. 1999, Chittaro and Hogan 2012), estuarine areas (Swearer et al. 2003, Carson et al. 2010), and open coast environments (Warner et al. 2005, Becker et al. 2007, Cook et al. 2014). Although the dispersal of planktonic larvae of benthic invertebrates has been studied systematically, the specific link between small-scale coastal geography and larval supply is less well resolved (Adams et al. 2014). In the eastern boundary of upwelling systems, sinuous coastlines and topographic features, such as the

presence of capes and associated bays, can influence the degree of population connectivity, through interactions between regional upwelling/downwelling processes and local-scale topography (Siegel et al. 2008). Elemental fingerprinting is increasingly being applied to understand connectivity patterns in complex environments given its potential to detect not only bay-open coast dispersal patterns (Becker et al. 2007, Sorte et al. 2013, Carson 2010) but also along-shore interchanges between populations in upwelling systems (López-Duarte et al. 2012). Coastal upwelling systems around the world have been extensively studied because of their high productivity and the physical mechanisms involved in along- and cross-shore larval transport (Roughgarden et al. 1988, Wing et al. 1995, Shanks and Brink 2005, Narvaez et al. 2006, Morgan et al. 2009). In the western Iberia upwelling ecosystem several studies have highlighted the importance of variability in the frequency and intensity of upwelling episodes to larval dispersal and recruitment of a diversity of invertebrate species (Queiroga et al. 2007, Peteiro et al. 2012, Nolasco et al. 2013). However, large scale studies on invertebrate larval dispersal pathways remain in the dominion of simulation modelling (Domingues et al. 2012, Nolasco et al. 2013), and might not reflect local scale connectivity patterns. Small scale topographic features can influence the degree of population connectivity, by generating different hydrodynamic stress amongst open coasts and protected embayments (Nicastro et al. 2008, Carson 2010).

Present theoretical frameworks and binding agendas at international (Convention on Biological Diversity) and European (OSPAR Commission, Marine Strategy Framework Directive) levels are advocating for the establishment of ecologically coherent MPA networks by 2020. Population connectivity is one of the four assessment criteria proposed to evaluate the degree of ecological coherence of systems of protected areas (Ardron 2008), with important implications for the persistence and resilience of metapopulations (Botsford et al. 2010). In Portugal, however, an estimate of population connectivity among MPAs is yet to be accomplished. Therefore, regional-specific scientific input on ecological patterns of connectivity, operating at a suitable temporal and spatial scale is crucial, if we are to deliver effective outcomes to established conservation policy targets. Here we focus on the central west coast of Portugal, which encompasses two Marine Protected Areas included in the European ecological network of protected areas Natura 2000. Although both MPAs in this study were initially established in a broad biodiversity conservation and fishery management context, single-species quantitative measurements of connectivity are important to identify the best range of reserve spacing which can maximize benefits for marine larvae with potential large-scale dispersal among habitat patches. *Mytilus galloprovincialis* has been largely employed as a model species to study connectivity patterns between subpopulations because of its broad distribution and its



function as an ecosystem engineer (Becker et al. 2007, Fodrie et al. 2011, López-Duarte et al. 2012). Our objective was to determine the spatial resolution of geochemical signatures in *Mytilus galloprovincialis* larval shells to reveal connectivity patterns between MPAs and adjacent areas. The complex topography of the coastline, characterized by prominent capes, bays and estuaries, represent an interesting setting for microchemistry-based investigations. Natural tags were investigated in a snapshot manner in summer 2013, using a large scale and short-term static atlas of elemental variability in mytilid larval shells. This reference map was employed to reconstruct the natal origin of newly settled mussels, under complex circulation patterns during the typical spring–summer upwelling season when northerly winds off Western Iberia usually prevail and cause upwelling of cold and nutrient rich waters (Relvas et al. 2007). We further integrate and compare the results with simultaneous environmental physical data, to assess whether the patterns we observed (geochemical fingerprints and dispersal pathways) were consistent with trace elemental composition, oceanography and hydrographic conditions of the area. Our results confirm the feasibility of the technique to accurately quantify self-recruitment and connectivity among MPAs, at ecologically relevant scales, within the complex coastal topography and bathymetry of the central Portuguese west coast.

## 2.2 Material and Methods

### 2.2.1 Species description

In Europe, the Mediterranean mussel (*Mytilus galloprovincialis*, Lamarck 1819) is distributed throughout the Mediterranean and along the Atlantic coast as far north as north-western Ireland (Gardner 1992). It was chosen as a model species as it is widely distributed in temperate marine rocky shores, making it particularly suitable to assess environmental-related signatures. Also, as an important structural component of rocky intertidal ecosystems, mussels play a key role as ecosystem engineers; increasing microhabitat complexity, environmental heterogeneity and benthic species richness with significant influence at the ecosystem-level (Borthagaray and Carranza 2007). Along the central coast of Portugal, mussels are subjected to an informal traditional fishery, depending largely on site accessibility, to supplement diet, for commerce or bait (Rius and Cabral 2004).

As broadcast-spawning invertebrates, fertilization occurs in the water column leading to a series of free-swimming planktotrophic larval stages (Bayne 1976). Shell mineralization starts  $\approx 20$ h after fertilization forming prodissoconch I which enlarges until the trochophore is completely enfolded forming the D-veliger (24-48h after fertilization; Ruiz et al. 2008). Primary

settlement sets the beginning of the juvenile form, and occurs when pediveliger larvae metamorphose and selectively anchor onto benthic surfaces by secreting byssal threads. The final step of settlement in bivalves is manifested after metamorphosis by a change in shell morphology and composition, with a differentiation of the prodissoconch (larval shell) and the dissoconch (benthic shell). Although planktonic larval development and duration (PLD) are strongly dependent on temperature and food availability, *M. galloprovincialis* larvae stay in the plankton for  $\approx 2$ -4 weeks (Ruiz et al. 2008), with the possibility to delay metamorphosis if suitable settlement substrates are not available (Chicharo and Chicharo 2000). Philippart et al. (2012) investigated the presence of mytilid larvae in European coastal waters as a proxy for time of reproduction and reported a seasonal pattern for the Iberian Coast with one major peak in spring and a less significant peak during the fall.

### 2.2.2 Area description

The study was carried out on rocky shores along the Portuguese central continental coast, an area delimited in the north and south by long sandy shores. This region incorporates major three-dimensional variations in coastline orientation and bathymetry (capes, bays and large estuaries) and its oceanography is complex, with recurrent wind stress variation and strong upwelling/downwelling seasonality (Relvas et al. 2007). Initially, and based on the coastal topography and oceanography, we have considered four main regions along the central west Portuguese coast. We separated northern and southern Estremadura branches, divided by the Peniche peninsula (Cape Carvoeiro) and delimited in the south by Cape Roca (Fig. 2.1). In Cape Carvoeiro, there are strong and recurrent wind stress variations, filament formation and separated coastal jets, suggesting the presence of recirculation cells, downstream of the capes (Oliveira et al. 2009). Cascais Bay and Arrábida Bay represent important discontinuities along the central Iberian west coast, more sheltered from upwelling prevalent winds, and under direct influence from two major estuaries (the Tagus and the Sado), whose basins drain heavy industrialized areas of Portugal.

The study area encompasses two Marine Protected Areas included in the Natura 2000 network. In Estremadura, the Berlengas Marine reserve is a coastal archipelago comprising three major islands, small islets and reefs. Arrábida Marine Park expands along 38 km of coastline, from just north of Cape Espichel to the mouth of the Sado estuary (Fig. 2.1).

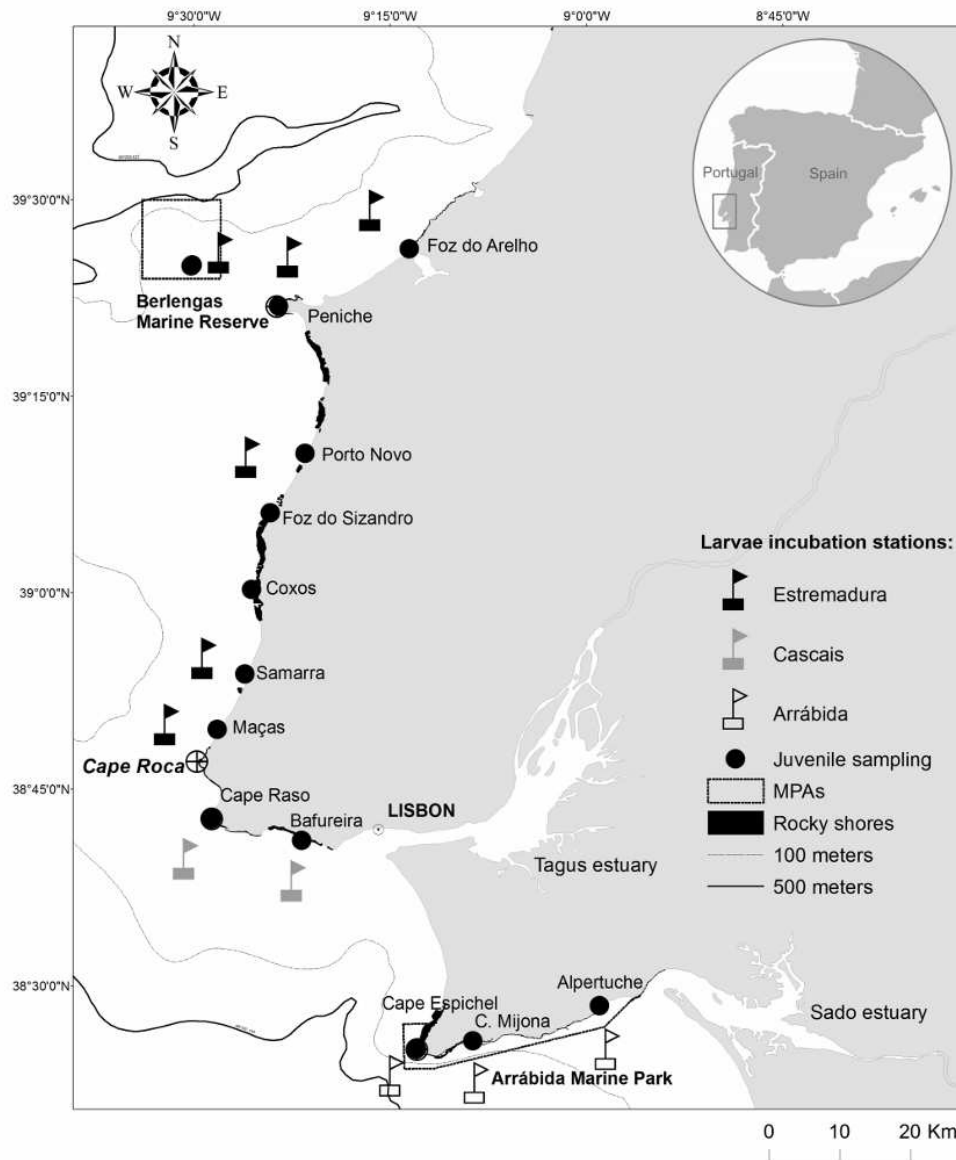


Figure 2.1 Map of larval incubation stations, juvenile sampling sites and location of Marine Protected Areas (MPAs). Estremadura North: Berlengas, Peniche and Foz do Arelho; Estremadura South: Porto Novo, Samarra, Maças; Cascais: Cabo Raso, Bafureira and Arrábida: Cabo Espichel, Cova da Mijona, Alpertuche. The final regions used to discriminate among natal regions were Estremadura (all moorings located north of Cape Roca), Cascais and Arrábida. For better visualization purposes, moorings in the map are illustrated more offshore than in the field (deployed at a depth of approx. 15-20m).

### 2.2.3 Mussel spawning and *in situ* larvae incubation

*M. galloprovincialis* wild adults were collected from the Costa Nova Naval Club pier (Aveiro, Portugal) in early June 2013, and were thoroughly cleaned and stocked dry at 4°C. Spawning was induced the following day by exposing the mussels to cyclic thermal stimulation (20 min at 25°C heated artificial seawater, followed by 20 min at 4°C) and spawning individuals were

confined to separate glass jars in order to collect the gametes independently. Eggs and sperm were filtered through a 125  $\mu\text{m}$  sieve and transferred separately to 250ml glass cylinders for quality check under a microscope. A small volume of the sperm solution ( $< 10\text{ml}$ ) was added to the egg suspension and carefully stirred to allow fertilisation. After 15 min the mixture was filtered using a 40  $\mu\text{m}$  sieve to remove excess sperm. Egg concentrations ranged from 350-1000 per ml. All tools, containers and pipettes were non metallic and subjected to acid leaching (50% v/v M $\Omega$  HCl 37%, HCl fuming 37% Emsure<sup>®</sup> grade, Merck, Germany) for 24hours, rinsed three times in Milli-Q water (Milli-Q 18M $\Omega$ ) and dried in a laminar flow chamber.

We followed the Becker et al. (2007) protocol for in situ larval incubation and used 25cm PVC pipe (500 ml inner volume) as larval incubators, with 41 $\mu\text{m}$  nitex mesh caps on each end. These incubators were washed in advance with Extran MA 03<sup>®</sup> 5% phosphate-free detergent for 1 day, leached in reverse osmosis water for 3 weeks (changing the water every 1-3 days) and acid-leached. Around 18000-20000 larvae (no shell, multi-celled embryos with less than 12h development) were split to each incubator and transported inside large buckets filled with artificial sea water to the incubation sites. All incubators were deployed in the water less than 12h after fertilization. Simultaneously, and in order to test for incubator effects on larval shell chemical signatures, we also reared larvae in the laboratory: two cultures loose in buckets and two cultures inside incubators. The cultures were fed *Isochrysis sp.* every two days and allowed to grow for seven days.

Eleven sites were selected in the central part of the Western Portuguese margin, offshore of known source of adult mytilid mussel populations. Along the very exposed coast, three sites (Foz do Arelho, Peniche and Berlengas) were located north, and three sites (Porto Novo, Samarra and Maças) south of Peniche. Two sites were situated in Cascais (Cabo Raso and Bafureira), and three along the Arrábida (Cabo Espichel, Cova da Mijona and Alpertuche) (Fig. 2.1). On the 4<sup>th</sup> and 5<sup>th</sup> of June 2013, two to three larvae incubators were deployed at each site, at a depth of approximately 3 to 5 meters, attached to a polypropylene cable that connected a signalling buoy to a concrete anchor block. Moorings were placed in 15-20 m of water and the buoys kept submersed (around 1.5m) to minimize theft and conflict with local fishers. One mooring, at Maças, was lost. The incubators were retrieved after six days in the water and were immediately filtered using local seawater, stored in acid-washed 120ml containers and frozen at  $-20^{\circ}\text{C}$ . This period allowed for larval shell development under exposure to local physical and chemical environmental conditions.

Early settlers of *M. galloprovincialis* were collected between 23<sup>rd</sup> and 25<sup>th</sup> July of 2013, approximately 43 days after the *in situ* incubation experiment to match the same planktonic development period as the incubated larvae. Three replicates of turf-forming algae were

collected at 13 sites (Fig 2.1) along the intertidal zone, inshore of the incubator deployment sites. Individuals < 1.5 mm in length were sorted in acid-washed glass petri-dishes under illuminated magnifying lenses, using Milli-Q water and Teflon coated extra fine forceps. Samples were frozen (-20°C) in acid-washed eppendorf vials.

#### **2.2.4 Mytilid larval and juvenile shell extraction and cleaning**

All shells prepared for geochemical analysis were processed using non-metallic acid-washed equipment, ultrapure reagents and Milli-Q water (reagents of certified trace metal purity 30% H<sub>2</sub>O<sub>2</sub>, 99% NaOH and 60% HNO<sub>3</sub> of Suprapur® grade, and HCl fuming 37% Emsure® grade, Merck, Germany). Larval shells were handled under a dissecting microscope, using the tip of a thin paintbrush. Complete shells were selected, separated and carefully placed in Milli-Q water drops. Shells were then transferred into cleaning solution droplets (15% H<sub>2</sub>O<sub>2</sub> buffered with 0.1 N NaOH) for 10 minutes, to remove all organic material, and rinsed 3 times in Milli-Q water, gently swiping the paintbrush in clean Milli-Q water in between relocations. Larval shells were transferred onto a gridded microscope slide that had been precoated with a thin layer of resin (Buehler's Epo-Thin™) and were embedded in a small bit of resin, using a fibre paintbrush bristle, to spread it over and around the larval shells, so that they were lying flat on the slide. Juvenile shells were prepared using the same methodology, but the valves were manually opened and split using the paintbrush after spending 15 minutes in the cleaning solution (heated in 60°C hot water bath). Shell length (larval and juvenile) was measured before embedding the samples onto resin (Buehler's Epo-Thin™) coated gridded microscope slides. Juvenile shells were positioned with the umbo facing upward.

#### **2.2.5 LA-ICPMS analysis**

Concentrations of trace elements in mytilid shells were determined using on an Agilent 7700 Inductively Coupled Plasma - Mass Spectrometer (ICP-MS) coupled to a HeEx (Laurin Technic and the Australian National University) laser ablation (LA) system with a 193nm Compex 110 (Lambda Physik) excimer laser. Random blocks of 18 samples each were run to avoid possible bias due to short-term instrument drift. Each block of samples was bracketed by runs of calibration standards spiked with trace elements (National Institute of Standards and Technology NIST 610 and 612) and a matrix-matched consistency standard MACS-3 USGS (U.S. Geological Survey MACS-3) for estimating external analytical precision (%RSD) (Table 2.1). Prior to each standard and sample analysis a 30 seconds blank was acquired to correct for background noise as to estimate the limits of detection of the method (Table 2.1). Both larval and juvenile shell microchemical composition was analysed individually using single spot laser

ablation (single crater; laser beam diameter = 32  $\mu\text{m}$ , laser energy = 60 mJ, laser repetition rate = 5 Hz). Newly recruited juvenile shells were ablated in the umbo region of the early prodissoconch (larval shell). The elements acquired were:  $^7\text{Li}$ ,  $^{11}\text{B}$ ,  $^{24}\text{Mg}$ ,  $^{31}\text{P}$ ,  $^{34}\text{S}$ ,  $^{39}\text{K}$ ,  $^{43}\text{Ca}$ ,  $^{55}\text{Mn}$ ,  $^{59}\text{Co}$ ,  $^{63}\text{Cu}$ ,  $^{66}\text{Zn}$ ,  $^{88}\text{Sr}$ ,  $^{111}\text{Cd}$ ,  $^{140}\text{Ce}$ ,  $^{208}\text{Pb}$  and  $^{238}\text{U}$ . For the larval samples, we only considered the readings which had at least 60000 counts of Ca, since many elements were below the detection limit of the method for samples with lower yields. For juvenile samples, and given the small size and orientation of the prodissoconch in the horizontal position of the shell, we restricted the data integration to include only the scans for the first two seconds of sample ablation. This was done to minimize contamination of the natal habitat signature as the laser burned through the early larval shell and into the underlying late-stage larval and juvenile shell (Strasser et al. 2007). This could be a potential problem with larvae that may spent only a short time at their natal location, influenced by strong currents or upwelling events, such as the area characterized in this study. Data were post-processed to remove any spikes (single scan values greater than two times the median of three adjacent scans) and smoothed (using a running average of 3 scans) to reduce the noise due to analytical imprecision. Standards and samples were blank subtracted and the abundance of trace elements standardized to molar ratios relative to calcium, to account for differences in the amount of ablated material.

Table 2.1 Detection limits (DL), percentages of samples above DL and precision estimates (% relative standard deviation, RSD) for the LA-ICPMS analysis of the larvae and juvenile shells. DL based on the blank analyses (18 per block of samples) and expressed in molar ratios relative to mean Ca concentration in a sample. External precision estimates based on %RSD using standards that most closely approximated the concentration of each element in a sample (MACS-3 used for all elements except for K and U, where NIST 612 was used).

Element	DL mol Ca <sup>-1</sup>	% above DL	%RSD
Li	10.49227 ( $\times 10^{-6}$ )	27	5.1503
B	0.08156 ( $\times 10^{-3}$ )	71	13.4084
Mg	0.00702 ( $\times 10^{-3}$ )	100	3.1578
P	0.17119 ( $\times 10^{-3}$ )	92	7.452
S	0.96897 ( $\times 10^{-3}$ )	82	7.5643
K	0.09088 ( $\times 10^{-3}$ )	93	12.4534
Mn	2.24422 ( $\times 10^{-6}$ )	59	2.5061
Co	0.55398 ( $\times 10^{-6}$ )	17	6.8978
Cu	1.5772 ( $\times 10^{-6}$ )	85	7.006
Zn	0.00317 ( $\times 10^{-6}$ )	100	7.8005
Sr	0.00412 ( $\times 10^{-3}$ )	100	4.3558
Cd	0.07271 ( $\times 10^{-6}$ )	41	5.6593
Ba	0.00044 ( $\times 10^{-6}$ )	100	3.6053
Ce	0	100	4.5512
Pb	0	100	7.0967
U	0.00003 ( $\times 10^{-6}$ )	100	2.6832

### 2.2.6 Environmental Data

A Daily Upwelling index at Cabo de Roca (UI;  $\text{m}^3\text{s}^{-1}\text{km}^{-1}$ ) was calculated from the 6-hourly data available (from the 1<sup>st</sup> June to 31<sup>st</sup> July 2013) by the Spanish Institute of Oceanography (Instituto Español de Oceanografía <http://www.indicedeafloramiento.ieo.es>). This index is calculated according to Lavín et al. (1991) for the Iberian Peninsula and using sea level pressure of the Meteogalicia WRF atmospheric model (<http://www.meteogalicia.es/modelos>). Daily Sea Surface Temperature (SST; °C) was averaged for each region (Estremadura, Cascais and Arrábida) from data provided by the HYCOM model (<http://www.hycom.org>) using the same configuration of Nolasco et al. (2013) with a 3 km resolution. HYCOM is a community ocean model which utilizes generalized vertical coordinates (Bleck 2002). Daily chlorophyll-a concentration for each region was averaged from Chl-a (mg. m<sup>-3</sup>) maps derived from MODIS data obtained from the Goddard's Space Flight Centre ocean colour data archive (NASA Goddard Space Flight Center, 2014).

### 2.2.7 Statistical Analysis

We started by analysing for any incubator effects on trace element concentrations in the larval shells raised at the laboratory using one-way ANOVA. Data were transformed ( $\log+0.01$  for all element ratios, but  $^{31}\text{P}:^{43}\text{Ca}$  which was 4<sup>th</sup> root transformed) in order to accomplish normality and homoscedasticity. Since a significant increase in concentration was found for ten trace elements in larval shells reared inside incubators, we proportionally subtracted that effect from the signatures of the larvae cultured in the field. We then performed a linear Discriminant Function Analysis (DFA) on the resulting element ratios ( $\text{X}:^{43}\text{Ca}$ ) to test the discrimination capability of multi-elemental fingerprints in larval shells among regions (Estremadura North, Estremadura South, Cascais, Arrábida). An analysis on geochemical differences among sites was not possible due to the small sample size at the site level. A forward stepwise analysis was employed to select the elements to build the discriminant functions ( $F$  to enter=1.5) and prior probabilities were computed taken into account group sizes. Reclassification success was evaluated using a jack-knifed classification matrix. A randomization method (White and Ruttenberg 2007) was used to assign p-values to jack-knifed reclassification success estimates and standardized canonical discriminant function coefficients were evaluated to assess the relative contribution of each trace element in calculating group assignment. One-way ANOVAs followed by post-hoc Tukey tests were performed to test the effect of region on the concentration of the element ratios introduced in the LDA functions. To determine whether our sampling effort was sufficient to capture variability within the 3 regions, based on Simmonds et al. (2014), we carried out linear discriminant function analysis using Monte Carlo cross-validation over different subsets of the data set (100%; 90%; 80%; 70% and 50% of the data) as implemented in the *mlr* library (Bischi et al. 2016) of R 3.2.5 (R Core Team, 2016 <https://www.R-project.org>). For each fraction of the original data set we performed 1000 iterations where data were randomly selected and misclassification error calculated. The larval shell discriminant function analysis was then used as a training set to assign recruits to natal origin. When assigning recruits to a natal source, DFA assumes all individuals to have originated from one of the three regions provided in the training dataset. As we did not sample all potential source populations, we assumed a conservative approach that any recruit assigned to a specific natal region with a probability of group membership <0.90 had an “unknown” origin. Finally, we calculated dispersal distance and direction for each successfully assigned individual (dispersal distance range were calculated from the collection site to the nearest and furthest point inside the natal region).



We considered self-recruits all individuals estimated to have settled into the same region where spawning took place.

## 2.3 Results

### 2.3.1 Larvae incubation and creation of an atlas of natal signatures

In situ mussel larval incubations yielded larval shells entirely formed at known locations, but survival rates inside the incubators were very low (<1%). The small size and fragility of the shells resulted in additional sample loss during shell extraction and cleaning. Sample numbers and average shell width ( $\pm 1$  standard error) were: Estremadura North  $n=21$ ,  $115.6 \mu\text{m} \pm 3.8$ ; Estremadura South  $n=11$ ,  $92.8 \mu\text{m} \pm 3.3$ ; Cascais  $n=15$ ,  $116.9 \mu\text{m} \pm 5.7$  and Arrábida  $n=16$ ,  $97.8 \mu\text{m} \pm 5.8$ . The larval shells of *M. galloprovincialis* showed differences in geochemistry that allowed us to separate them using linear discriminant function analysis. Grouping the incubated larval shells by site resulted in low accuracy of assignment (average of 43.7% of cross-validated cases correctly classified). The assignment accuracy increased at the regional level (Estremadura North, Estremadura South, Cascais and Arrábida regions), with an average of 68.3% of cross-validated cases correctly classified (Fig. 2.2A, Table 2.2). However, Estremadura North and Estremadura South regional-specific geochemical signatures showed linear discriminant scatterplots with overlapping group centroids (Fig. 2.2A, Functions 1 and 2 of group centroids = -1.5, 0.4 and -1.4 and 0.4, respectively) with low (40%) cross-validated classification success in the Estremadura South region (Table 2.2). Therefore we decided to combine both locations into one single open coast region (Estremadura) and rerun the LDA (Fig. 2.2B). The resulting cross-validated classification success increased to 79.5% (Table 2.2), significantly higher than the 33.0% expected by chance alone ( $p = 0.0002$ , White and Ruttenberg 2007). All the subsequent analyses were performed using three natal source regions- Estremadura, Cascais and Arrábida.

Classification accuracy was highest for larvae incubated in the Estremadura region (90.6%) while larvae reared in Cascais had the lowest classification accuracy (60.0%) (Table 2.2).

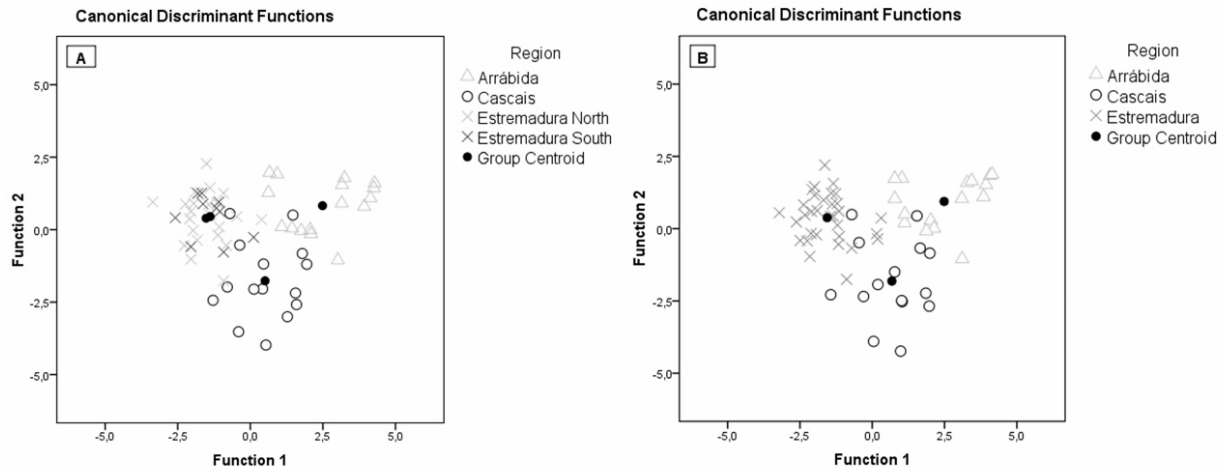


Figure 2.2 Canonical score plots of the linear discriminant function (LDF) analyses for incubated larval shells of *M. galloprovincialis* (4<sup>th</sup>-11<sup>th</sup> June 2013), by regions. Each data point represents one shell; regions are represented by a separate symbol and color. In (A) we used our predefined regions based on local topography and oceanography while (B) represents LDF analysis using three regions.

Table 2.2 Jack-knife classification success of DFA of incubated *M. galloprovincialis* larval shells, with sampling sites grouped into A) 4 regions and B) 3 regions. Correct classifications are in bold (average of 68.3% and 79.5% of cross-validated cases correctly classified, when considering 4 and 3 regions respectively, n= number of individuals).

Predicted Group Membership (%)				
Region (A)	Arrábida	Cascais	Estremadura North	Estremadura South
Arrábida (n=16)	<b>75.0</b>	12.5	12.5	0.0
Cascais (n=15)	20.0	<b>60.0</b>	20.0	0.0
Estremadura North (n=22)	4.5	4.5	<b>81.8</b>	9.1
Estremadura South (n=10)	0.0	20.0	40.0	<b>40.0</b>
Predicted Group Membership (%)				
Region (B)	Arrábida	Cascais	Estremadura	
Arrábida (n=16)	<b>75.0</b>	12.5	12.5	
Cascais (n=15)	20.0	<b>60.0</b>	20.0	
Estremadura (n=32)	0.0	9.4	<b>90.6</b>	

Eight trace elements entered the model (B, P, Co, Cu, Zn, Ce, Pb, and U) and the first canonical function explained 73% of total variance, with Pb and Zn, B, P with positive loadings and Cu, Co, Ce, U with negative loadings (Table 2.3). The second canonical function explained the remaining 27% of total variance, with Zn and Co loading negatively. Arrábida and Estremadura were separated mostly by the first function, with positive values for Arrábida and negative values for Estremadura, indicating higher concentrations of Pb and P in Arrábida. The second function separated Cascais from the other two regions, with higher values of Zn in this region.

Table 2.3 Standardized canonical discriminant function coefficients corresponding to the canonical score plot in Fig.2.2B. Percentages of variance and canonical correlation coefficients for each function are also shown.

Molar Ratios	Function	
	1	2
$^{11}\text{B} : ^{43}\text{Ca}$	.484	.353
$^{31}\text{P} : ^{43}\text{Ca}$	.708	.486
$^{59}\text{Co} : ^{43}\text{Ca}$	-.558	-.323
$^{63}\text{Cu} : ^{43}\text{Ca}$	-.735	.088
$^{66}\text{Zn} : ^{43}\text{Ca}$	.209	-1.024
$^{140}\text{Ce} : ^{43}\text{Ca}$	-.246	.254
$^{208}\text{Pb} : ^{43}\text{Ca}$	1.271	.268
$^{238}\text{U} : ^{43}\text{Ca}$	-.644	.342
<b>% of Variance</b>	73	27
<b>Canonical Correlation coefficient</b>	.868	.729

Univariate analyses of variance comparing ratios to calcium for the 8 elements used to discriminate larval shells among regions resulted in significant differences in four elements (Table 2.4, Pb:Ca  $F=24.463$   $p=0.00001$ , Zn:Ca  $F=11.501$   $p=0.000059$ , U  $F=0.961$   $p=0.000086$  and Ce  $F=3.878$   $p=0.026065$ ). Larval shells incubated in Cascais had significantly higher concentrations of Zn and lower concentrations of Ce compared with the other two regions; for Pb concentrations: Arrábida>Cascais>Estremadura; and for U concentrations:

Estremadura=Arrábida>Cascais (Fig. 2.3). Mean misclassification error to the region level (3 regions: Arrábida, Cascais, Estremadura) derived from the full model and from the cross-validation with 10%, 20%, 30% and 50% of the data withheld were then compared using an ANOVA ( $F=19.71$ ,  $p<0.001$ ). Tukey HSD post-hoc tests confirmed no significant differences in misclassification error between the full model and those subsets which included at least 80% of the data. The consistency detected on classification success among those subsets confirmed our capability to detect distinctive signatures for each region and sufficient sampling effort to account for variability within each region.

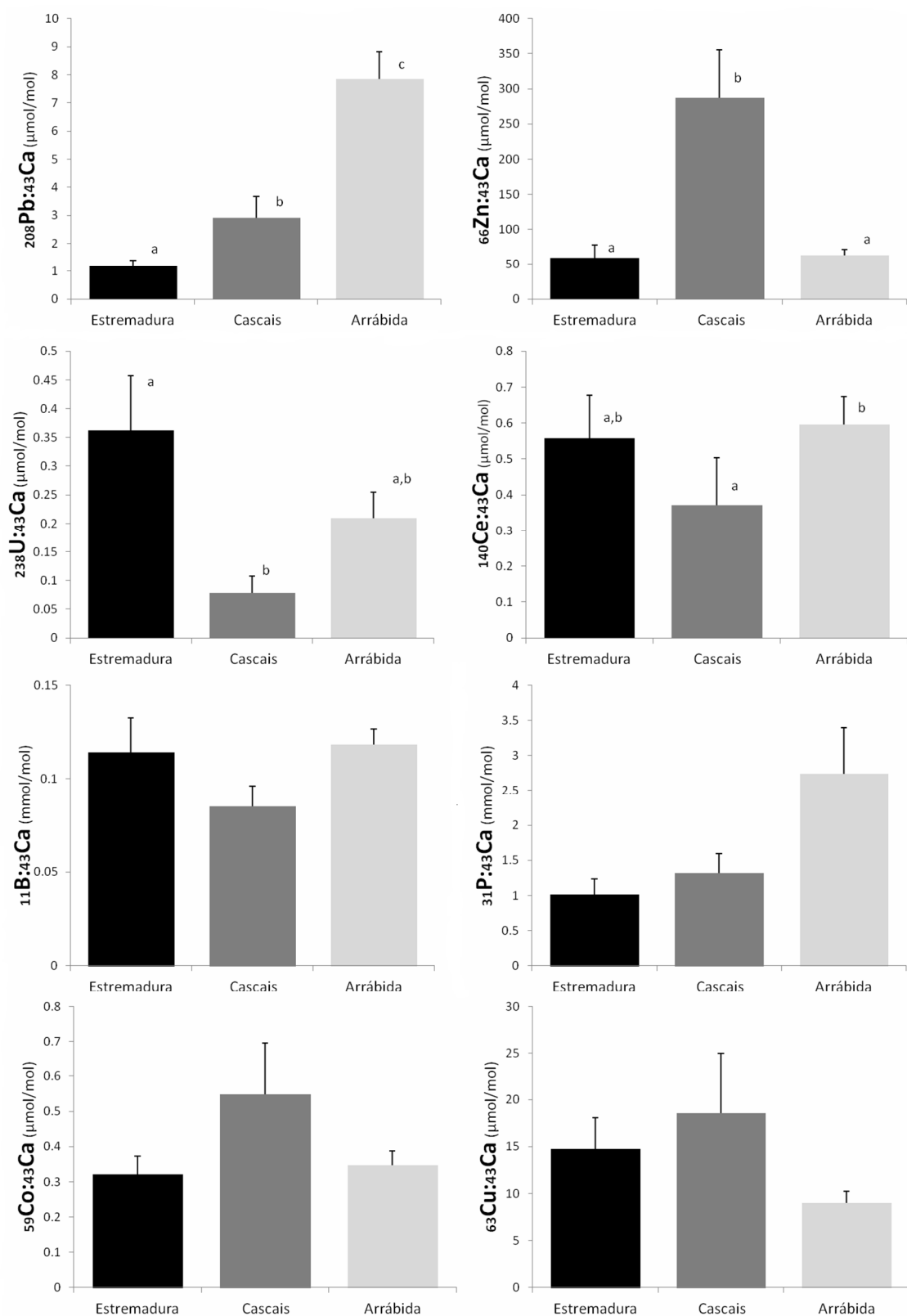


Figure 2.3 Ratios to calcium for the 8 elements used to discriminate larval shells of *M. galloprovincialis* from the regions Estremadura, Cascais and Arrábida. Bars represent the regional mean with 1 standard error bars. Letters above bars indicate significant differences according to Tukey post-hoc tests.

Table 2.4 Univariate analysis of variance of the effect of region (Estremadura, Cascais and Arrábida) on trace element concentrations in larval shells of *Mytilus galloprovincialis*. Only trace elements which entered the LDF model were included in this analysis. Bold values indicate significant effects at the 5% significance level. df= degrees of freedom; SS= sum of squares; MS= mean square; F<sub>s</sub>= F test value; p= probability value. Post hoc pairwise comparisons using Tukey tests are shown in Fig. 2.3.

<b><sup>208</sup>Pb:<sup>43</sup>Ca</b>					
Source of variation	df	SS	MS	F <sub>s</sub>	p
Region	2	5.513	2.756	24.463	<b>0.00001</b>
Error	60	6.250	0.104		
Total	62	11.763			
<b><sup>66</sup>Zn:<sup>43</sup>Ca</b>					
Source of variation	df	SS	MS	F <sub>s</sub>	p
Region	2	4.319	2.160	11.501	<b>0.000059</b>
Error	60	11.267	0.188		
Total	62	15.586			
<b><sup>238</sup>U:<sup>43</sup>Ca</b>					
Source of variation	df	SS	MS	F <sub>s</sub>	p
Region	2	1.922	10.980	0.961	<b>0.000086</b>
Error	60	5.252	0.104		
Total	62	7.175			
<b><sup>140</sup>Ce:<sup>43</sup>Ca</b>					
Source of variation	df	SS	MS	F <sub>s</sub>	p
Region	2	1.244	0.622	3.878	<b>0.026065</b>
Error	60	9.622	0.160		
Total	62	10.866			
<b><sup>11</sup>B:<sup>43</sup>Ca</b>					
Source of variation	df	SS	MS	F <sub>s</sub>	p
Region	2	0.161	0.080	2.343	0.105
Error	60	2.056	0.034		
Total	62	2.216			
<b><sup>31</sup>P:<sup>43</sup>Ca</b>					
Source of variation	df	SS	MS	F <sub>s</sub>	p
Region	2	0.241	0.136	1.766	0.180
Error	60	4.612	0.077		
Total	62	4.883			
<b><sup>59</sup>Co:<sup>43</sup>Ca</b>					
Source of variation	df	SS	MS	F <sub>s</sub>	p
Region	2	0.216	0.108	1.062	0.352
Error	60	6.096	0.102		
Total	62	6.312			
<b><sup>63</sup>Cu:<sup>43</sup>Ca</b>					
Source of variation	df	SS	MS	F <sub>s</sub>	p
Region	2	0.380	0.190	1.062	0.352
Error	60	10.732	0.179		
Total	62	11.111			

### 2.3.2 Establishment of natal origin of juveniles

128 recent settlers of *M. galloprovincialis* were collected in the Estremadura region at 8 sites (average width  $\pm 1$  standard error,  $462.7 \mu\text{m} \pm 31.18$ ), 30 in Cascais, at 2 sites ( $833.3 \mu\text{m} \pm 172.35$ ) and 43 individuals in Arrábida, at 3 sites ( $549.57 \mu\text{m} \pm 30.37$ ). The collection occurred approximately 42 days after the incubation period. The small size of the individuals assembled ( $534.23 \mu\text{m} \pm 49.01$  SE) indicates that settlement had occurred in the preceding two-three weeks. With a larval duration of approximately 3-4 weeks for this species, at the temperatures recorded during the study, the larval incubation period matches the early stages of planktonic larval development for these recruits. Out of 201 juvenile mussels, 81 (40.3%) were allocated an unknown origin (probability of group membership  $<0.90$ ), most of them collected from Estremadura (72.8%). When “relaxing” our criteria for successful recruit assignment based on the posterior probabilities in the DFA, the overall percentage of individuals from “unknown origin” drops from 40.3% ( $<0.90$ ) to 24.4% ( $<0.75$ ) and to 5% ( $<0.5$ ). In all three scenarios, however, the general pattern in larval dispersal distance and direction remains the same. For this reason, and because we want to account for the inherent uncertainty in the atlas of natal signatures, we have presented the most conservative approach in larval assignment and discuss the likely origins of unknown individuals based on local oceanographic and topographic characteristics. Within a region, the natal origin of the recruits collected was variable, mainly for the Estremadura region, where recruits exhibited greater diversity in natal sources (Fig. 2.4), suggesting high heterogeneity in the local hydrodynamics. Recruits collected in the bays of Cascais and Arrábida, primarily fall under the domain of Arrábida natal signature, accentuating the high self-recruitment within its bay, high larval export from Arrábida and no self-recruitment amongst Cascais bay, for this period.

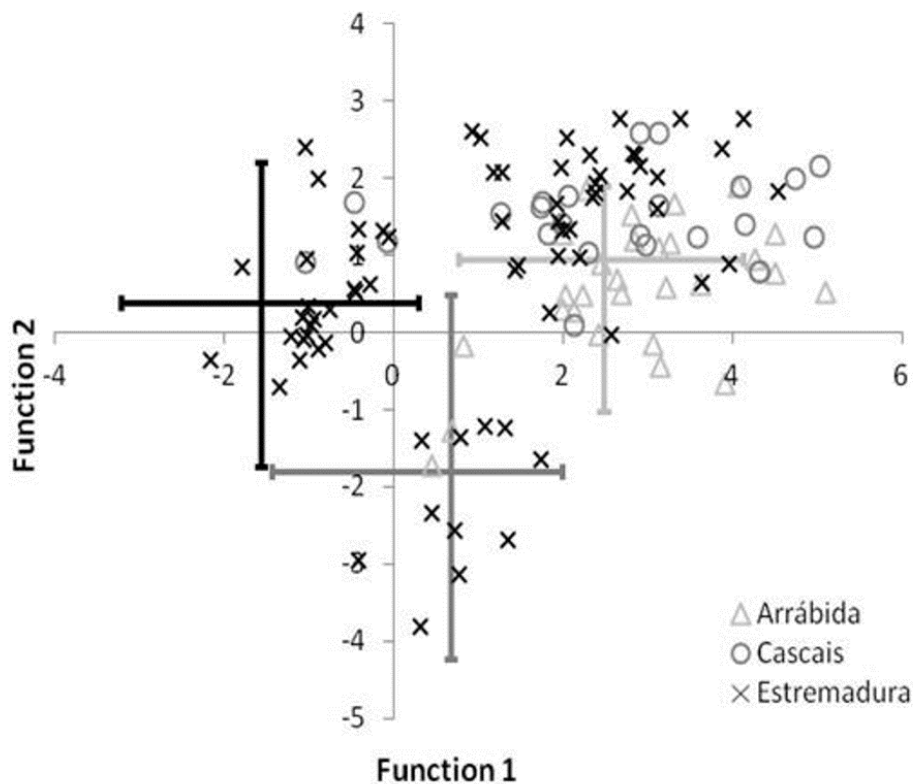


Figure 2.4 Canonical score plot of the individual recruits according to the discriminant analysis based on larval shell elemental signature. Symbols represent where recruits were sampled. Lines represent average larval scores (centroid) and extent (maximum and minimum values) for each region, following the colour code: light grey, dark grey and black for Arrábida, Cascais and Estremadura, respectively. Recruits which fell under the 90% confidence interval for assignment are not shown.

We found evidence for mussels in Estremadura to have originated from Arrábida, with recruits collected as far as Baleal and Berlengas estimated to have dispersed more than 100 km north (Fig. 2.5). Also, recruits collected in Estremadura originated in Cascais (9%) and self-recruitment was detected within the region (19% of juveniles collected in the Estremadura region originated in that region) (Fig. 2.6). Cascais showed no self-recruitment and appeared to be the region with least importance in larval export to other regions. Interestingly, 70% of all recruits collected in Cascais came from Arrábida Bay (which is an MPA). Arrábida MPA showed the greatest contribution as a source population, and high levels of self-recruitment (58%), with only few recruits originating from Cascais (5%) and none from the most northern region, Estremadura (Fig. 2.6).

Natal origins of recruits in the Berlengas MPA were the most diverse, with little connectivity to Arrábida MPA. In terms of dispersal direction, 55.4% of the reassigned recruits originated from southern natal sources and only 4.1% were supplied from northern locations, which clearly



indicate a northward dispersion pattern (Fig. 2.7). Regarding distances, most of the recruits analyzed here were estimated to have dispersed less than 50 km away from the natal source, with maximum dispersal ranges of about 120 km.

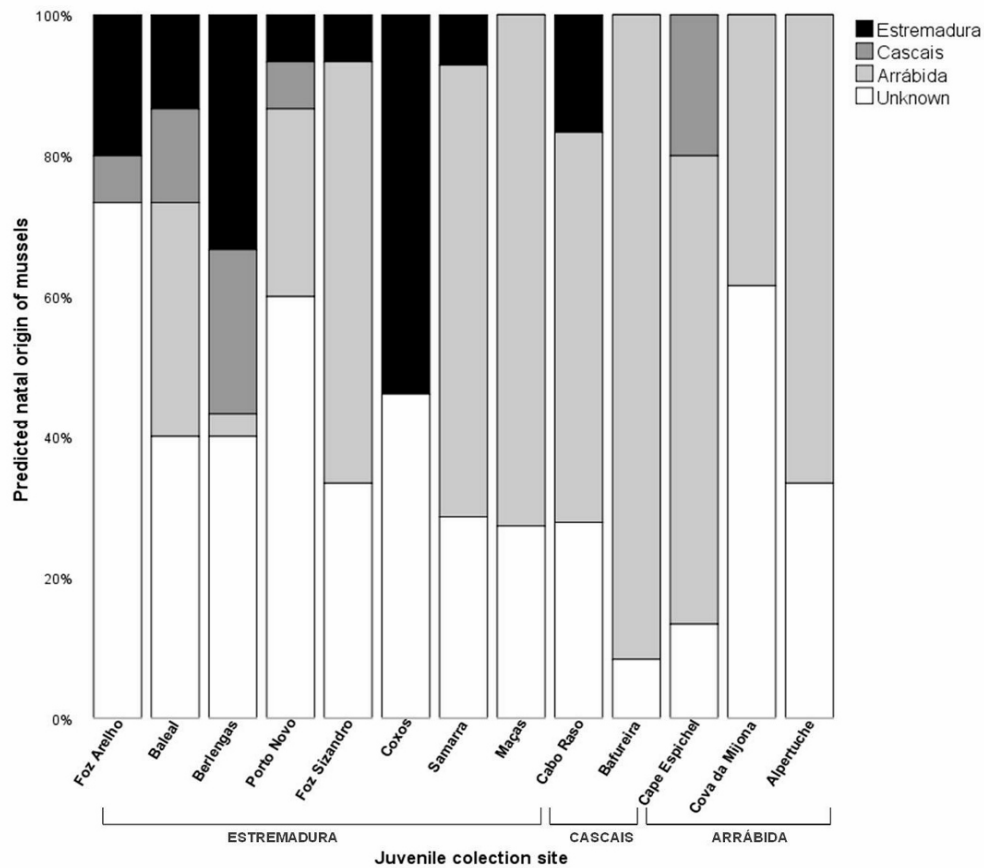


Figure 2.5 Predicted natal origins of juvenile mussels. X axis represent collection sites of juvenile mussels (early settlers), grouped by main regions, and the colors of the bars symbolize predicted natal origins determined by using regional larval shell DFA functions as a training set. Sites are organized from North (left) to South (right). See Fig. 2.1 for a site map.

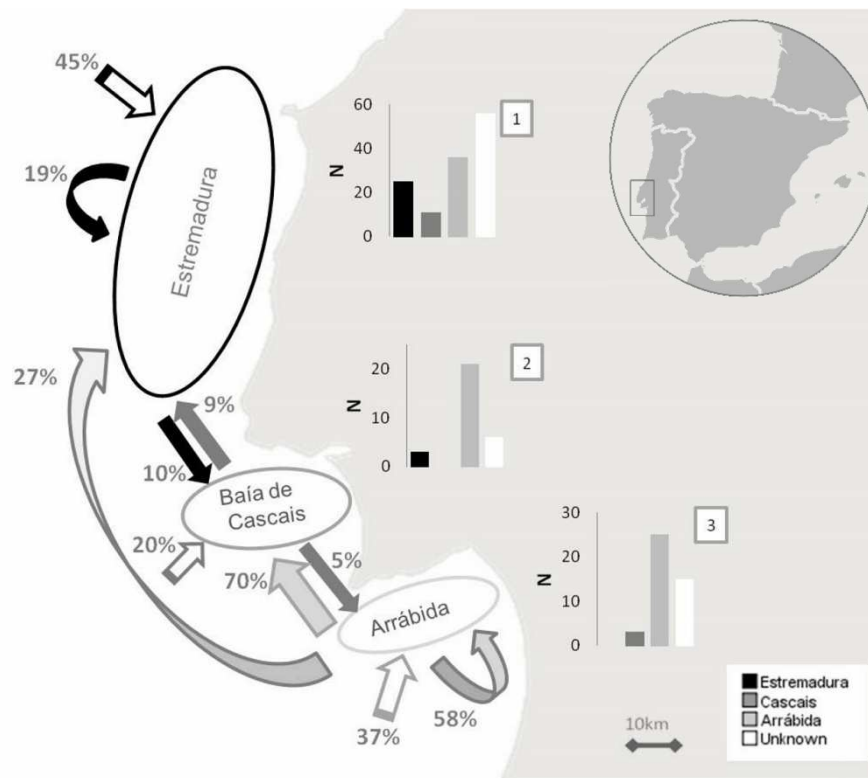


Figure 2.6 Predicted dispersal pathways of *Mytilus galloprovincialis* larvae among the three regions in the central west coast of Portugal, during mid June to mid July 2013. Arrow's color and width represent recruits which originated from the region at the base of the arrow. White arrows indicate unknown origin. Bar graphs 1, 2 and 3 correspond to the number of juveniles collected (by region) and predicted natal origins (by region), for Estremadura, Cascais and Arrábida correspondingly.

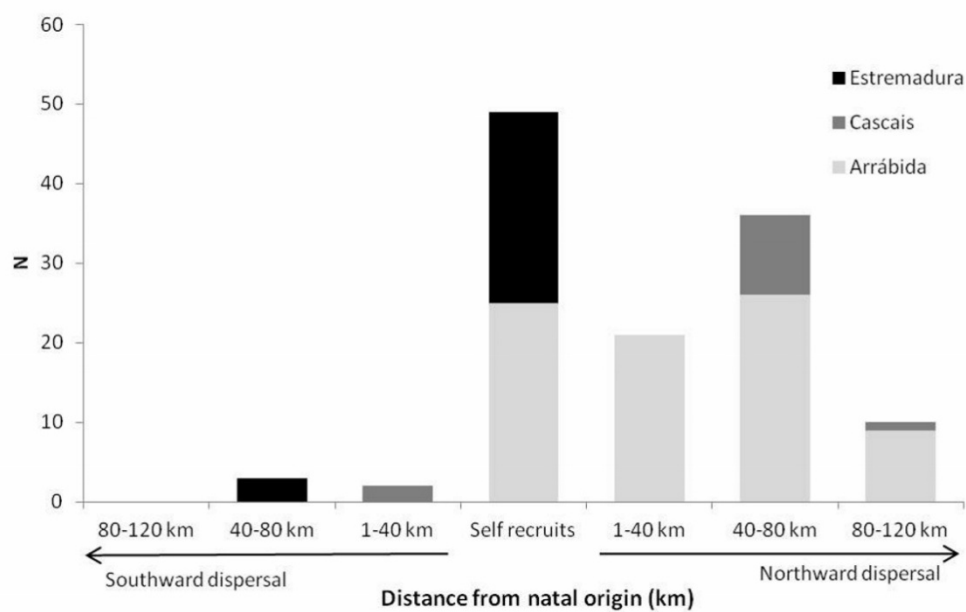


Figure 2.7 Predicted dispersal direction and distance from natal source (km) of *Mytilus galloprovincialis* successfully assigned recruits (n = 121), during mid June to mid July 2013. Self-recruits are individuals estimated to have settled into the same region where spawning took place.

### 2.3.3 Environmental Data

The daily Upwelling Index series (Fig. 2.8) from June and July 2013 at Cabo da Roca showed a strong upwelling event from the 15<sup>th</sup> of June until the 3<sup>rd</sup> of July, followed by an extensive relaxation period which lasted until the 24<sup>th</sup> of July. This long relaxation period (3 weeks) is unusual for this season, which is typically characterized by prevailing northerly winds (Relvas et al. 2007). An abrupt SST warming took place immediately after the upwelling maximum on the 23<sup>rd</sup> of June, rising from average values oscillating around 14.5 °C in June 21<sup>st</sup> to average values of 18.8 °C on July 11<sup>th</sup>, and stayed high until the end of July. Daily chlorophyll-a concentrations increased from around 2 mg/m<sup>3</sup> before the upwelling event to between 6 and 8 mg/m<sup>3</sup> immediately after the upwelling maximum, stayed high during the event and decreased again to around 2 mg/m<sup>3</sup> during the relaxation event. These temporal patterns are consistent with a northward advection of a warm water mass starting around the 25<sup>th</sup> of June, about 15 days after the larval incubation trial, and continued until the sampling of the recruits from the 23<sup>rd</sup> to the 25<sup>th</sup> of July.

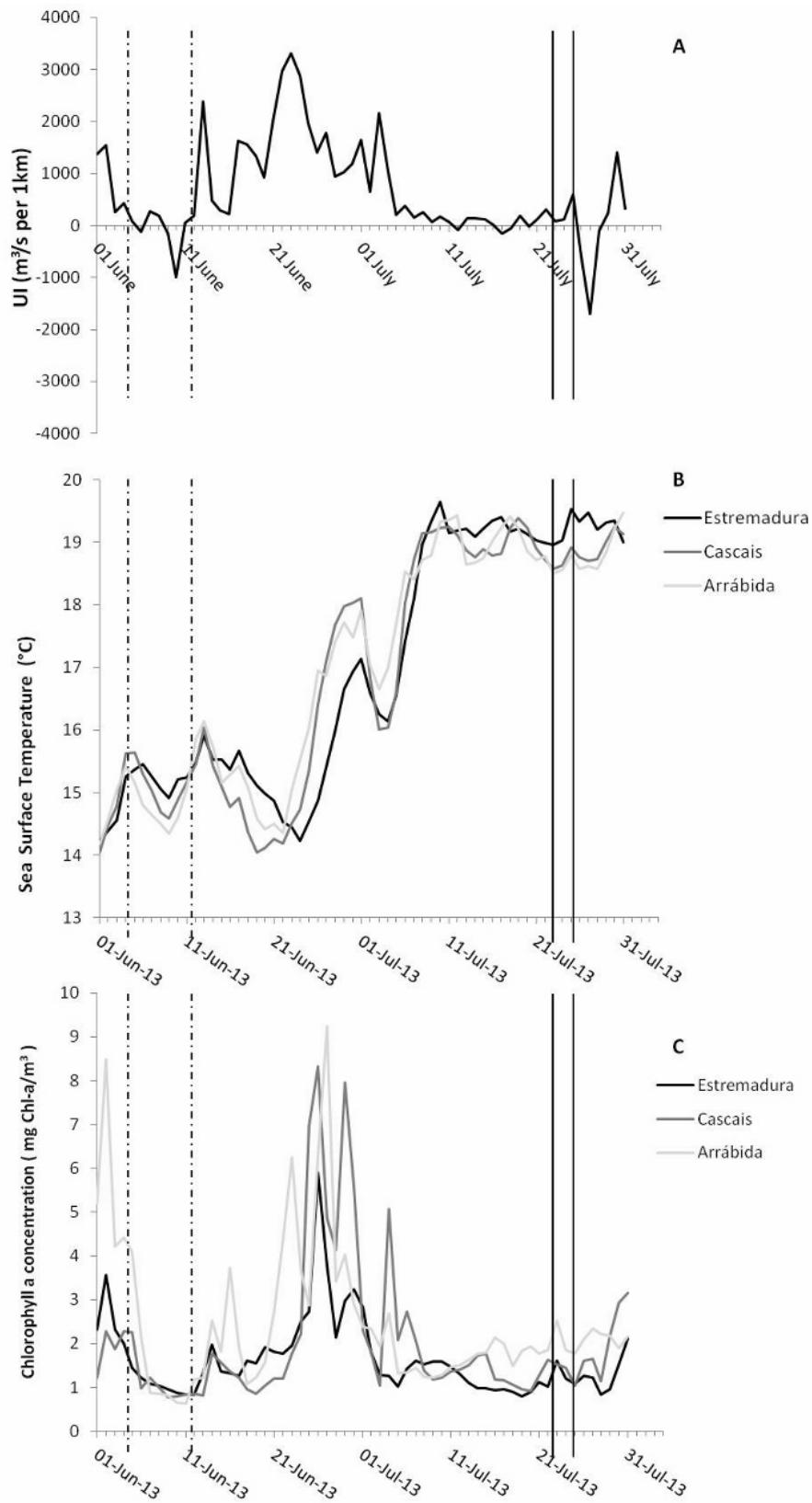


Figure 2.8 Daily time series of A) the Upwelling Index (UI) estimated for the Cabo de Roca, B) Surface Temperature (SST;  $^{\circ}\text{C}$ ) and C) Chlorophyll-a concentration averaged separately for each region, during June and July 2013. In A), negative values indicate downwelling. Larval incubation and recruit sampling periods are indicated in the graph by dashed and solid lines respectively.

## 2.4 Discussion

### 2.4.1 Larval-shell geochemical signatures

In this study, we provide further evidence that *M. galloprovincialis* larval shell geochemistry can disclose valuable information as environmental and natal tags at an ecologically relevant spatial scale, crucial in tracking larval dispersal pathways. *M. galloprovincialis* larvae reared at different sites along the central Portuguese west coast showed distinctive trace elemental signatures at the regional level, discriminating between the open coast from two large embayments exposed to industrialized estuaries. Distinctive elemental signatures in the biogenic carbonate from invertebrate larvae between open coast and bay habitats have been described before (Becker et al. 2005, Carson 2010, Fodrie et al. 2011). Trace element incorporation in biominerals can be influenced by a variety of factors, such as elemental concentrations in local seawater, and seawater temperature, salinity, and pH (Campana 1999, Chittaro et al. 2006, Levin 2006). During the incubation experiment, however, at least sea surface temperatures and salinities from all sites were very similar across the studied area (temperature values ranged from 14.75°C to 15.81°C, and salinity varied between 35.93 and 36.09). Although the incorporation mechanisms involved in the site-specific trace elemental composition in larval calcified structures is still uncertain for most elements, this knowledge is not required to successfully apply the technique for tracking natal origins (Gillanders 2002, Zacherl 2005, Becker et al. 2007, Carson 2010, Cook et al. 2014). In our study, zinc (Zn), lead (Pb), and uranium (U) concentrations on mytilid larval shells were the main variables responsible for discriminating regional signatures. These elements are amongst the group of useful elements (Mg, Cr, Mn, Co, Cu, Zn, Sr, Cs, Ba, Pb and U) reviewed by Carson et al. (2013) in southern California, in which the variation in the environment is usually reflected in teleost fish otoliths, bivalve shells and crustacean larvae. Both estuaries in our study area (the Tagus and the Sado) have been extensively documented as having increased anthropogenic trace metal concentrations, from urban wastewater, agricultural runoff and industrial effluents, in adjacent waters, suspended particulate matter, surface sediments and sediment cores (Caeiro et al. 2005, Costa et al. 2011, Santos-Echeandía et al. 2012). Our results showed that larvae reared inside the Cascais bay had higher concentrations of zinc in the shell. This is consistent with high trace metal concentrations in the surface waters and sediments in the Tagus estuary due to the effluents from chemical, steelwork and shipbuilding industries (Cotté-Krief et al. 2000). Arrábida Bay larval shells contained the highest concentrations of lead. Richter et al. (2009) also found anthropogenic Pb stable isotope signatures in sediment cores of the Setúbal-Lisbon canyons system, consistent with fly ash inputs from waste incinerators, and an efficient

transfer from the river discharge to the adjacent shelf. Pb has been reported as an effective marker in mussel shells in relation to polluted bays (San Diego Bay; Becker et al. 2005). Pb incorporation in the shell is frequently well correlated with water Pb concentration (Carson et al. 2010, Fodrie et al. 2011) and one of the elements showing less temporal variability in the open coast (Fodrie et al. 2011).

Along the exposed coast of Estremadura, larval shells showed significantly higher concentrations of uranium. Recently, the incorporation of uranium in biogenically precipitated carbonates has received some attention as a potential acidification geochemical proxy in foraminifera, corals, and mollusk larval shells, as reviewed in Levin et al. (2015). In order to probe for a geochemical proxy that reflects pH exposure in mussel larval shells, Frieder et al. (2014) cultured in the lab *Mytilus galloprovincialis* across a range in pH and temperature, and confirmed that U/Ca incorporation reflected mean pH conditions in the water, following a strong negative correlation, regardless of larval shell size, oxygen concentration or temperature. Additionally, the authors successfully applied that proxy to larvae reared along a spatial gradient in upwelling in Southern California, detecting higher U/Ca ratios in larval shells reared in colder, low pH waters. Similarly, in the exposed Estremadura region, CO<sub>2</sub> enriched and low-pH upwelled waters, might explain the higher U/Ca when compared to the contiguous and more protected bays of Cascais and Arrábida. However, we were unable to find pH values for this region and period to test for this hypothesis.

Although we found spatially distinct multielemental signatures, we do not know if the signatures are temporally stable. However, studies have suggested that trace element compositions within newly recruited bivalve shells (Becker et al. 2005) and larval shells (Cathey et al. 2014) can be relatively stable over weekly to monthly timescales. Even when signatures are temporally variable, spatial discrimination using bivalve shell chemistry is still often possible (Fodrie et al. 2011, Carson et al. 2013). The elements responsible for regional discrimination in this study are likely associated with consistent environmental differences among locations (i.e. strong upwelling exposed coasts versus bays influenced by urbanized estuaries). Nonetheless, the complexity of the shoreline, variable ocean circulation, and the dynamic nature of atmospheric and hydrologic pollution inputs actively influence and modify seawater geochemistry in coastal and estuarine systems (Swearer et al. 2003, Thorrold et al. 2007, Miller et al. 2013). Also, more studies are needed to fully understand how the material and environment inside artificial incubators can indeed interfere with the element uptake into the larval shell carbonate matrix. The potentially different elemental signature of incubator vs wild shells could hinder the recruit's assignment to natal origins. Further work should consider the use of diverse incubators, of different sizes and materials, and different larval densities and

parenting pools, in order to improve in situ rearing settings and to advance our understanding of the formation of geochemical signatures under “caging conditions”. Nevertheless, we are confident our results from the larval housing units were able to record regional variation in source signatures, between the open coast and two urbanized bays, where the environmental factors have prevailed over any regional maternal effects, or any effect due to leaching of elements from the incubators. This work represents a momentary and potentially transitory atlas of chemical fingerprints; a “snapshot” of the local physical, chemical and oceanographic characteristics between June and July 2013.

#### **2.4.2 Dispersal pathways vs. local oceanography**

When we assigned early mussel settlers to source populations across 120 km of coastline in the central west coast of Portugal, we were able to quantify natal origin and dispersal trajectory for 59.7% of the collected settlers. Within a region, the natal origin of the recruits collected was variable, mainly for the northern Estremadura region, where the number of “unknown origins” was also greater, which might suggest high heterogeneity in the local hydrodynamics in this open coastal setting. There were, however, two major sources of uncertainty included in the model: recruits that originated from outside of our study region (even though our sampling was delimited by long sandy- shorelines, a non suitable habitat for mussels) and recruits that originated from within our study region, but the signature from the source location was less consistent. For these reasons, we followed a conservative approach and only assumed successful recruit assignment when the probability (posterior probabilities in DFA) was  $> 0.9$ .

Bivalves have been described as a potentially long-dispersing species, with estimated dispersal distances reaching to 100s of kilometers (Bayne 1976). McQuaid and Phillips (2000) calculated that the majority of recruits of *Mytilus galloprovincialis* in South Africa settled  $< 5$  km from the parent population. Genetic studies (Kinlan and Gaines 2003), genetics and physical oceanography (Gilg and Hilbish 2003), trace elemental fingerprinting (Becker et al. 2007) and spatial geostatistical analysis (Smith et al. 2009) have also documented moderate dispersal distances (20–40 km) among open coast mussel populations. Lopez-Duarte et al. (2012) also reported along-shore dispersal distances of about 35 km for *M. californianus* and 37 km for *M. galloprovincialis* between generalized regions of origin and destination, in southern California. Accordingly, and even though this study revealed larval exchange among regions separated by more than 100 km, for most of the recruits analyzed the dispersal distance was estimated to be less than 50 km away from the natal source. However, the dispersal range analysis was constrained by the regional resolution of the natal signatures since geographical distances

within and amongst natal regions diverges. Nevertheless, it illustrates how far larvae can disperse under the local upwelling/ relaxation events.

In terms of dispersal direction, we observed an overall northward dispersal. The Estremadura region, which is much larger than the other two regions and has the most adult mussel habitat, contributed only 1.5% to other regions. This northward overall dispersal direction was unexpected since spring and summer periods are characterized by upwelling favorable winds resulting in southward ocean surface circulation over most of the shelf (as reviewed by Relvas et al. 2007). However, wind-stress reversals and upwelling relaxation events are common along the west Iberian coast at short temporal scales (days), affecting nearshore circulation (Relvas and Barton 2005, Oliveira et al. 2009). Upwelling relaxation events are well described along the eastern boundary of upwelling systems. These events, where the wind forces relax after a coastal upwelling event, have been associated with an increase in nearshore alongshelf poleward flow reversals in California (Melton et al. 2009, Send and Nam 2012) and Chile (Narváez et al 2006). The upwelling index, recorded in the region during June-July 2013 revealed the presence of an upwelling event followed by an extended upwelling relaxation period, with a sharp increase in SST and a decrease in chlorophyll-a concentration. Thus, it is possible that the newly formed mussel larvae were initially pulled southward (as a results of the upwelling event), but were then transported northward along the coast in July. Based on a multi-year observational study, Sordo et al. (2001) reported that, upon cessation of upwelling events, a northward flow progressed inshore along the Western Iberian northern margin, causing a narrow band of warm water against the coast. Also, Oliveira et al. (2009), using satellite images and numerical simulations of SST and Chl-a, reported a rapid onset of coastal counter currents along the inshore zone during upwelling relaxation, with northward flow of oligotrophic waters from Arrábida Bay and occupying part of Cascais Bay. Accordingly, 70% of the recruits we collected in Cascais were supplied by the Arrábida MPA. We found no evidence for self-recruitment in Cascais Bay. The hydrodynamics in this bay are strongly influenced by the Tagus estuary, one of the largest in Europe, whose plume can be advected offshore during upwelling favorable winds, and pushed back northward along the Estremadura coast during relaxation periods (Vaz et al. 2009).

#### **2.4.3 Implications for management and future directions**

Quantifying connectivity among coastal populations and identifying critical habitats to the replenishment of adult populations is crucial for assessing current spatial management approaches and to set the scale for future integrated management plans. Different methods to derive connectivity estimates differ in their specific objectives, and/or temporal resolution,



varying from integrative to snapshot assessments. Long term modeling studies have shown that larval connectivity is inherently a stochastic process varying as a function of different biological and physical processes (Siegel et al. 2008). Although this study derived connectivity estimates from a snapshot approach, such empirically-derived metrics are crucial to validate the predictions of coastal connectivity and resource dynamics from larger scale modeling efforts (Werner et al. 2007).

The Arrábida MPA management plan approved in 2005 imposed prohibition to trawling, dredging and bivalve harvesting, to preserve its role as nursery for many marine species contributing to the sustainability of the local fishing resources (Cunha et al. 2014). Our results showed that this MPA was the main source population supplying larvae to the other two regions, even though connectivity with Berlengas MPA was very limited. Arrábida MPA also contributed to 70% of the recruits collected in Cascais and revealed 58% of self-recruitment within its bay, suggesting a retention zone for locally spawned larvae. Other studies in the Arrábida Marine Park have also showed that fish larvae (namely reef-associated species belonging to the families Gobiidae, Tripterygiidae, Labridae and Sparidae) can complete their entire planktonic phase in the vicinity of the adults' habitats (Borges et al. 2006). Interestingly, Nicastro et al. (2008) studying *Perna perna's* gene flow in South Africa, showed that coastal topography strongly affected larval dispersal and population genetic structure, with bays acting as source populations. However, different reproductive seasons (spring and fall) along with changes in upwelling intensity might result in different dispersal trajectories for mytilid species (Carson et al. 2010).

Self-recruitment and connectivity via larval dispersal has been documented by several authors in the assessment of MPAs: using hydrodynamic (Roberts 1997), biophysical (Cowen et al. 2006) and spatial metapopulation models (White et al. 2010), genetics (Palumbi 2004), dispersal distances (Shanks et al. 2003), parental analysis (Planes et al. 2009) and more recently elemental fingerprinting (Di Franco et al. 2012, Cook et al. 2014). Here, we provide evidence for high self-recruitment within Arrábida MPA for mytilid larvae but limited connectivity with Berlengas MPA, during an upwelling relaxation event. Our results give further emphasis on the need to incorporate dispersal pathways and the variability in the local oceanographic setting when developing management plans regarding MPA placement and size.

Recently, Burgess et al. (2014) underlined the significance of local retention (the fraction of offspring produced by a population that also recruits into that population) rather than self-recruitment for the dynamics and persistence of spatially structured populations within MPA networks. In this sense, larval dispersal patterns require knowledge of larval production rates

to truly evaluate population dynamics and metapopulation persistence. Our next goal is to integrate and combine our results with numerical models of ocean circulation and population dynamic models, in order to have a more complete picture of what drives mytilid population dynamics and persistence in and around a network of MPAs, over larger temporal and spatial scales. Such direct measures of demographic connectivity can be a powerful tool used by field practitioners and policy-makers to refine monitoring programs and reassess the configuration of current reserves to deal with the contemporary issue of MPA network ecological coherence along complex topographic and oceanographic coastlines.

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## Chapter III

## What's a picture really worth? On the use of drone aerial imagery to estimate intertidal rocky shore demographic parameters

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**Abstract**

Describing the distribution patterns of organisms on the rocky intertidal zone is crucial to generate ecological models of broad scope and validity. Our aim was to combine 3D photogrammetric models and spectral analysis derived from aerial images, with ground-based quadrat sampling to provide realistic measurements of mussel (*Mytilus galloprovincialis*) density, size and reproductive output. A remotely piloted aircraft was used to conduct intertidal photographic surveys during low tides (<0.4m) over 8 study sites along the Portuguese central west coast. At each site, low altitude (30m) and high resolution photographs were collected (82 to 247 airborne photographs, 80% overlap, with a ground resolution of approximately 0.8 cm/pixel), encompassing an average intertidal area of 15200 m<sup>2</sup> per survey location. Images were mosaicked, georeferenced and a 3D photogrammetric model was constructed for each location. An analysis of the spectral signature for the different ground cover types was used to perform a maximum likelihood supervised classification with overall high classification accuracy (86.5%  $\pm$  4.3%, mean $\pm$ SD). Additionally, we analysed the effect of environmental variables (substrate complexity and wave exposure) on mussel density on rocky substrate and size, and found a significant effect of wave exposure in winter. Maximum mussel density occurred at intermediate values of wave exposure while smaller sized mussels were found at high values of wave exposure. Density, size and reproductive output maps were predicted for the study region. This information may support important metapopulation models dealing with the persistence of spatially-structured populations within patchy habitats and assist in the management of pivotal conservation areas.

**Keywords:** UAVs, rocky intertidal, photogrammetry, image classification, spatial metrics

*Mytilus galloprovincialis*

### 3.1 Introduction

As anthropogenic pressures in the coastal zone continue to rise (Boesch et al. 2001, Thompson et al. 2002), there is a growing need to resourcefully monitor, detect and forecast ecological patterns and processes to assist large-scale conservation strategies.

In this sense, the use of remote sensing, including aerial pictures, satellite images and acoustic data has provided the opportunity to produce large spatial and temporal datasets while decreasing physical or biological disturbance (Nagendra 2001, Kerr and Ostrovsky 2003, Wang et al. 2010). However, there is still a considerable disproportion between pixel resolution and the scale of ecological-relevant features and processes (Philipson and Lindell 2003, Zharikov et al. 2005) associated with high altitude photography. The required image spatial extent and resolution (ground size and pixel size, respectively), the spectral resolution (wavelength range), the frequency of image collection and the cost, are therefore important factors to consider when planning for airborne imagery datasets.

Regarding intertidal zones, one major challenge in ecology studies concerns not only the spatial extent for which data needs to be collected, but also the logistical challenges of surveying small organisms reaching very high densities. In addition, sampling is constrained to appropriate low tide and wave agitation intervals. Thus, theoretically, intertidal surveys should consist of fast assessments and cover large spatial areas with the maximum taxonomic resolution possible.

Historically, researchers have used field-based sampling and experimental ecology to monitor species assemblages and interactions in the intertidal zone (e.g. Paine 1974, Underwood 2000, Menge et al. 2015). The commonly used quadrat sampling is a precise and efficient method to survey benthic biological communities allowing the identification of species at high taxonomic resolution. However, they might misrepresent large scale patterns of species distribution and community structure. On the other hand, ground photographic surveys (e.g. Witman et al. 2004) are time-efficient and provide long term records (Reimers et al. 2014) but these images only provide reliable information on the most abundant and visible taxa (Godet et al. 2009).

For broader-scale intertidal mapping and monitoring, aerial imagery from satellite remote sensing or manned aircraft platforms have been commonly used (reviewed in Godet et al. 2009). Also, multispectral LiDAR surveys have been used to evaluate the distribution and structural complexity across large spatial scales in coastal habitats (Collin et al. 2012). Yet, the low resolution associated with these techniques often fails to detect small-scale patterns of heterogeneity in topography and detail in species abundance and distribution (Meyer et al. 2015).

The improvement of high resolution aerial imagery, following the progress in unmanned aerial vehicles (UAV, here referred as drones), offers novel possibilities for the scientific community (Watts et al. 2012, Anderson and Gaston 2013). The use of drones creates numerous advantages in environmental science: in the study of canopy and vegetation dynamics, precision agriculture, ecosystem processes, natural disaster management and spatial ecology (as reviewed in Anderson and Gaston 2013 and Shahbazi et al. 2014). In the marine and/or aquatic environment, drones' applications have focused on habitat characterization (Chabot and Bird 2013), coastal erosion (Quater et al. 2014), mapping and monitoring vegetation (Zaman et al. 2011, Strecha et al. 2012, Turner et al. 2014), characterizing water bodies' bathymetry (Lejot et al. 2007), thermal properties (Wawrzyniak et al. 2013) and topography (Mancini et al. 2013, Pérez Alberti et al. 2014). In addition, drones are being used in marine wildlife research, on the monitoring of waterbird colonies (Grenzdörffer 2013, Ratcliffe et al. 2015), marine mammals (Schoonmaker et al. 2008, Hodgson et al. 2010, 2013, Koski 2010, Mejias et al. 2013) and Antarctic predators (Goebel et al. 2015). Flight duration and safety, declining operational costs and increasing autonomy, fine spatial resolution and increased survey revisit periods, as well as aptitude to fly below the clouds and possibility to approach animals, are amongst the advantages of unmanned platforms.

This way, low-altitude and high resolution aerial photography offer a better trade-off between spatial coverage and image resolution, crucial for intertidal monitoring surveys. There is an increasing number of UAVs remote sensing studies focusing on intertidal rocky habitats, commonly considered an ideal "natural laboratory" and currently one of the most vulnerable marine ecosystems to anthropogenic stressors (Crowe et al. 2000, Halpern et al. 2007). Rocky reefs provide important and complex environments with sharp environmental gradients and exposure to wave action. Moreover, covering a large portion of the world's coastline, they grant important ecosystem services (Duarte 2000) and serve as a sentinel for the impacts of climate change (Helmuth et al. 2006). Studies of low altitude aerial photography of rocky intertidal areas vary from balloon platform to study topography and algal biomass (Guichard et al. 2000), to kites acquiring aerial multi-spectral photographs to construct high-resolution, photo-textured terrain models (Bryson et al. 2013). Very recent studies reveal the potential of drones in intertidal reefs to accurately monitor dominant algal communities (Murfitt et al. 2017) and the distribution patterns and patchiness of seagrass (Konar and Iken 2017). Also, drones have been successfully used for monitoring rocky intertidal boulder position over time to investigate disturbance in Northern Spain (Pérez-Alberti and Trenhaile 2015).

Aerial photography from piloted aircrafts has been used to study large-scale patterns of mussel distribution in intertidal mud and sand flats (e.g. Stoddart 2003, Herlyn 2005, Dolch and Reise 2010). Also, Barrell and Grant 2015 successfully used a low-altitude aerial photography from a helium blimp platform to study the spatial arrangement of a bivalve-macrophyte mosaic at an intertidal flat. However, to our knowledge, no published work aimed to specifically quantify mussel distribution in rocky shores using drone aerial photography.

The Portuguese west coast presents numerous intertidal rocky reefs harbouring rich biological communities, with mussels occurring in the lower mid-shore zone (Boaventura, Ré, et al. 2002). *Mytilus galloprovincialis* is a key ecological species influencing the biodiversity of intertidal ecosystems, sheltering, supporting and enhancing a diverse number of invertebrate species. Mytilid mussel beds or patches of mussels layers form a mussel matrix habitat structure essential to the maintenance of local species diversity by increasing spatial complexity and creating spatial refuges from environmental stresses (temperature, light, wave force) and predation (e.g Borthagaray and Carranza 2007). Also, as filter feeders, mussels regenerate nutrients into benthic biomass, benefiting algae growth and deposit feeders and sustaining intertidal food webs (Menge et al. 1997). This way, understanding the factors driving mussels' abundance, growth and productivity can provide important answers about the dynamics of the entire ecosystem, and help to develop models predicting population performance at larger scale and under different environmental scenarios.

Wave action is recognized to be an important environmental factor influencing biological communities in the rocky intertidal ecosystems (Stephenson and Stephenson 1972) mainly affecting growth (Mcquaid et al. 2000, Denny and Wethey 2001), fertilization (Pearson and Brawley 1996), larvae settlement (Hunt and Scheibling 1996), dislodgement and size distribution (Mcquaid et al. 2000, Hunt and Scheibling 2001). For sessile marine species, such as mussels, population survival depends of trade-offs between high nutrient and larvae supply and increased chance of dislodgement on very exposed shores (Steffani and Branch 2003).

Here, we aim to combine low altitude aerial images with 3D topographic information (from digital elevation models) and ground-based quadrat sampling to quantify mussel (*M. galloprovincialis*) coverage, density and size (and consequently reproductive output) along the Portuguese central west coast. Moreover, we tested the effect of two major abiotic factors known to influence mussel distribution: wave exposure and substrate complexity. The effect of wave exposure was calculated using a fetch index combined with local wind data (as described in Burrows et al. 2008).

We explicitly address the following research questions:

- i. Are low altitude high resolution aerial images useful in investigating fine-scale rocky intertidal topography and mussel coverage in rocky intertidal shores?
- ii. Does combining aerial images with 3D photogrammetric models and ground-based quadrat sampling provide realistic measurements of mussel bed coverage area?
- iii. Are wave exposure and terrain roughness good explanatory variables of mussel density and size?

Our results support several emerging studies which integrate low altitude multispectral imagery with high resolution surface models and ground based observations, as a viable and powerful tool for surveying topographic complex intertidal ecosystems. Fast, efficient and cost-effective methods producing large scale estimates on species distribution patterns are crucial to assist competent conservation management strategies dealing with species persistence.

### **3.2 Material and Methods**

#### **3.2.1 Study area and species description**

This study was carried out on the rocky shores along the Portuguese west central coast (38°N - 40°N). The study area incorporates major variations in coastline orientation and topography, including capes, submarine canyons, sandy and rocky shores, bays and estuaries (Fig. 3.1). It includes very exposed areas, subjected to strong seasonal upwelling (Relvas et al. 2007) and more protected sites from prevailing north and northwest winds (Fig. 3.1). At minor spatial scales, the intertidal landscape is highly variable at the scale of metres, with a semidiurnal tidal regime ranging up to 4m. In the North-East Atlantic Ocean, summers are characterized by small and short-period waves from northern mean directions while winters have more energetic wave conditions, such as larger and longer-period waves from south-west to north-west mean directions (Dodet et al. 2010).

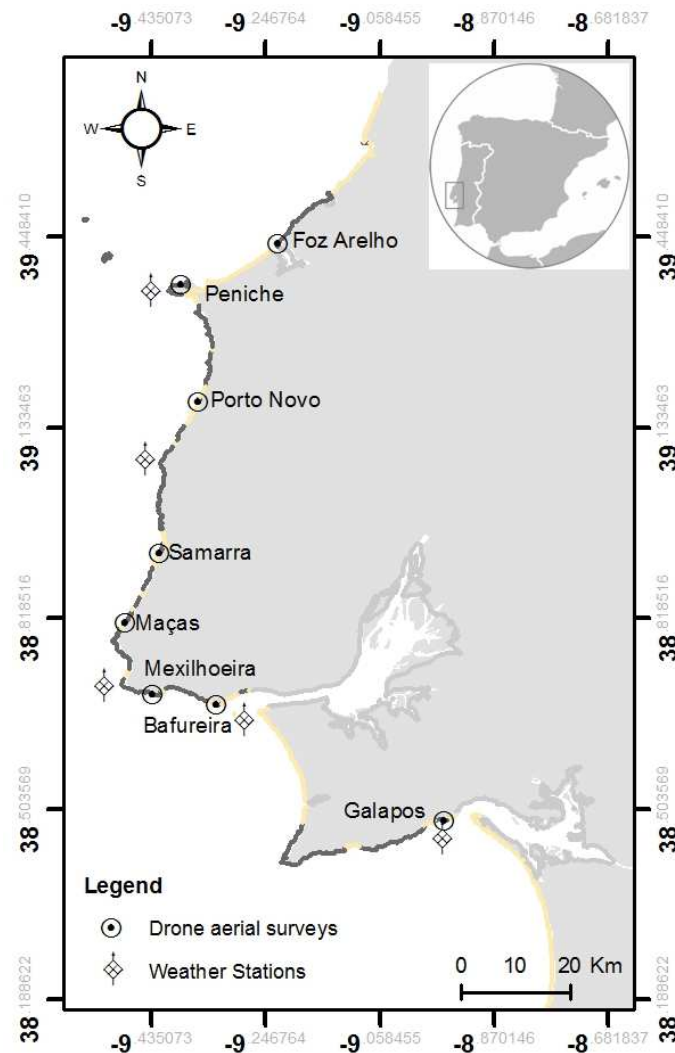


Figure 3.1 Map of the central west Portuguese coast showing the location of the drone aerial surveys and the weather stations (from North to South: Cabo Carvoeiro, Santa Cruz, Cabo Raso, Praia da Rainha and Setúbal. The coastline colour scheme represents: sandy shores (yellow), rocky shores (dark grey) and estuaries (light grey).

*Mytilus galloprovincialis* is an important ecological component of worldwide temperate rocky shore communities, native to the Mediterranean (Hilbish et al. 2000). As an ecosystem engineer, mussel beds cause physical changes in the substrate and associated biota, influencing the availability of resources to other species (Gutierrez et al. 2003, Borthagaray and Carranza 2007). *Mytilus spp.* disperses only via planktonic larvae (3 to 4 weeks, Ruiz et al. 2008) following a benthic sessile adult phase. Along the Portuguese coast, distribution patterns of *Mytilus galloprovincialis* have been described in the mid and low-shore intertidal communities, along wave-exposed and bay habitats (Boaventura, Re, et al. 2002, Araújo et al. 2005). Mussels are subjected to an informal but intense traditional fishery, depending largely on site accessibility, to supplement diet, for commerce or bait (Rius and Cabral 2004).

### 3.2.2 Drone flight, imagery and model methodology (workflow summarized in Figure 3.2)

#### 3.2.2.1 Image acquisition

The Aerial surveys were performed by a trained pilot using a V-Form Octocopter-drone manufactured by Ascending Technologies GmbH, Krailling, Germany (model AscTec Falcon 8). The drone had attached a NEX 5N Sony digital camera (23.4x15.6mm), 16.1 MP Exmor™ APS HD CMOS image sensor. A mobile ground station allowed real-time management of flight control, information (data link, remote camera control, video connection, status display and telemetry) and data recording. Flight and data acquisition were planned in advance based on the study area and the required image resolution and size. After conducting a test flight to obtain practical information of the photogrammetric results of the images at different heights, we decided to keep the aerial surveys at an altitude of approximately 30m.

Weather conditions varied over the 5 days of flights, from clear to partly cloudy with low winds. Aerial images were collected from 8th – 12th September 2014, at the intertidal zone, during very low tides (<0.4m) at the following sites in the Portuguese central west coast: Samarra, Porto Novo, Peniche, Mexilhoeira, Bafureira, Maçãs, Galapos, Foz do Arelho. Sites were selected from available aerophotogrammetric coverage of the Portuguese coast (<http://mapas.igeo.pt/lidar/>) to ensure representative rocky shore locations and were classified according to their morphometry into two categories: Boulders or Flat Platform. Flights were pre-programmed inserting GPS tracks over survey areas and the drone performed fully automated low altitude (around 30m) flight routes by waypoint navigation, producing high resolution images with 80% overlapping areas. A minimum of 6 and a maximum of 12 ground survey quadrates (20x20cm) were haphazardly placed on the mussel beds, within the three biological intertidal zones, high, mid and low, identifying presence or absence of mussels. For each quadrate, GPS coordinates (handheld Garmin GPSMAP 62S) and digital photographs were taken to calculate percentage of mussel coverage at each quadrat using Image J® 1.50i. All the mussels inside the quadrats were collected and refrigerated. In the lab, mussels were separated, counted and photographed in a white tray. All the mussels present in each quadrat were measured along their antero-posterior axis using Image J® 1.50i analysis software, and the average length (Length; L) was calculated.

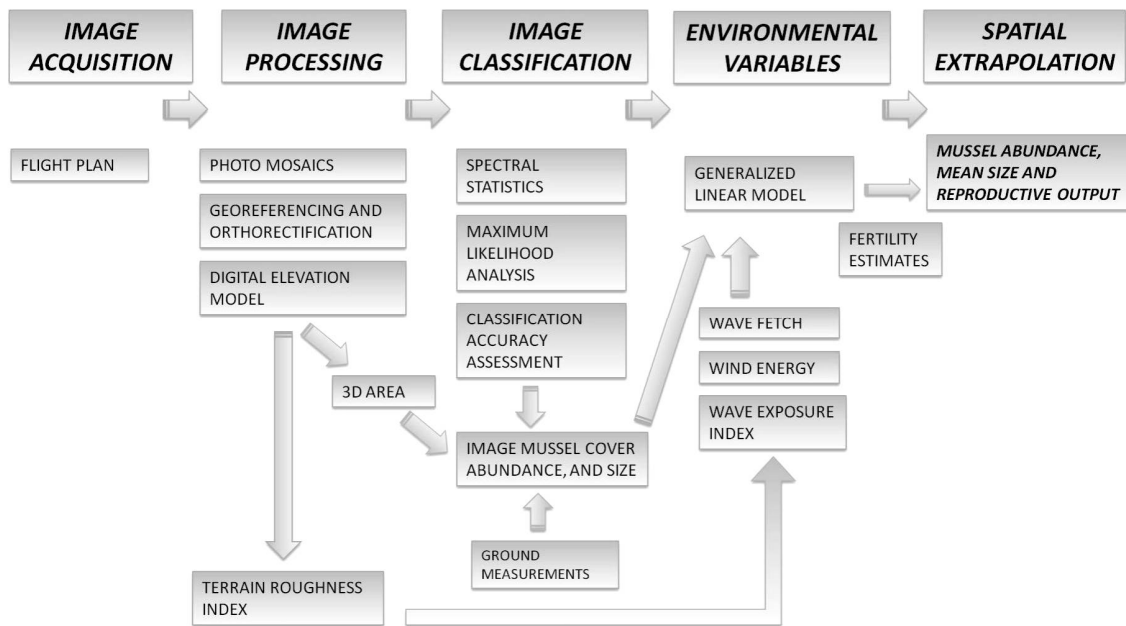


Figure 3.2 Workflow summarizing the steps followed in the flight planning, image acquisition, processing and spatial extrapolation of mussel abundance, size and reproductive output in the study area.

### 3.2.2.2 Image processing

In order to generate high-resolution geo-referenced orthophotos, we used Agisoft PhotoScan Pro software for photogrammetric processing of digital images. The software combines airborne GPS data (camera latitude, longitude and altitude), with point-cloud technology and compensates for intensity/color differences between overlapping images during the assemblage of the photo mosaic. The software camera alignment uses the aerotriangulation (AT) method, which determines the image position and rotation in space. A pair of overlapping images is oriented to one another by measuring the same ground object (matching points) in each of the corresponding images, generating a sparse point cloud. Using collinearity equations to identical points in multiple images (Yuan et al. 2009), the software is then able to determine any point location on the ground, in relation to the images using space intersection (the ground x, y, and z location) to project each image pixel to its corresponding ground location on the model. A dense point cloud is created and used to re-construct the 3D geometry (interpolating the points into a 3D polygonal mesh). The textured geometry can be exported as a digital elevation model (DEM), representing the model surface as a regular grid of height values.



### 3.2.2.3 Image Classification

ArcGIS Spatial Analyst was used for supervised image classification. This process assigns each image pixel to pre-defined thematic classes, converting multiband raster imagery into a single-band raster composed by a number of classes.

For each mosaic, we selected the main terrain cover classes: water, sand, rocks, mussels, algae and/or urban, depending on the landscape characteristics. Since the purpose of this study was the quantification of mussels, we used generalized classes for vegetation. In some locations, each individual class was also divided into shadow and light, or wet and dry categories, to account for the environmental variability effect on the image. For each class, we selected representative 100+ training samples (small polygons of about 20cm<sup>2</sup>, covering the entire image extent) from the high resolution image, zoomed to the maximum. This way, each training set characterized the typical pixel spectral pattern of the terrain-cover classes.

The classification analysis is sensitive to the range of values in each band and works on the assumption that the band data in the training set follows a normal distribution. For this matter, the spectral statistics of individual training classes were explored, visualizing the RGB histograms and scatterplots for each class, to ensure normal distribution and enough separation between classes. Depending on the outcome of the training set evaluation, training samples were re-edited and reevaluated to ensure representativeness of classes. The software then creates a parametric signature for each class, generating a spectral signature file for each mosaic. Finally, we used the Maximum Likelihood Classification algorithm which makes use of a discriminant function to determine the membership of the pixels in the mosaic to each class represented in the signature file.

Each pixel was then assigned to the class with the highest likelihood or left unclassified if the probability values were all below a threshold of 0.05. The resulting classified image is composed of a collection of pixels, color-coded to represent each class.

### 3.2.2.4 Classification Accuracy Assessment

Accuracy assessment of classified images helps to evaluate the quality of classification obtained. Here, we define accuracy as the degree of correspondence between the classification and visual references in the image, and expressed it by means of error matrices. Error matrices compare the relationship between known reference data and the corresponding results of the classification procedure, on a class-by-class basis.

In this sense, we selected reference samples (validation polygons) in the high-resolution image in order to project them on the classified image and perceive the relationships between the real and the classified image. Validation data was composed of evenly distributed small

reference polygons for each class (between 100 to 150 polygons, with 5-10 pixels each), which we could visually identify from the original high-resolution photographs. To guarantee that the reference data for the class “mussels” was the most accurate as possible, we selected the polygons inside the field ground quadrats easily discernible when zooming in the photos.

For each image, an error matrix was created to express a quantitative accuracy assessment of class membership (Stehman and Czaplewski 1998). It is represented by contingency tables where diagonal entries characterize conformity between the classified pixel class and reference data, and off-diagonal entries represent misclassifications. We evaluated the following accuracy parameters: overall accuracy, Cohen’s kappa coefficient and probability of commission and omission errors (Table 3.1).

Table 3.1 - Definition of the evaluated parameters in the error matrices (adapted from Stehman and Czaplewski 1998).

<b>Overall accuracy</b>	Represents the overall proportion of the area correctly classified, and is calculated by dividing the total correct assignments (the sum of the major diagonal) by the total number of pixels in the error matrix.
	Estimates overall accuracy, indirectly taking into account the omission and commission errors. It varies from 1 and 0 (perfect agreement or randomness between model prediction – classified image – and reality, respectively). Kappa is computed as
<b>Kappa coefficient</b>	$K = \frac{N \sum_{i=1}^r x_{ii} - \sum_{i=1}^r (x_{i+} * x_{+i})}{N^2 - \sum_{i=1}^r (x_{i+} * x_{+i})}$ <p>where N is the total number of observations in the matrix, r is the number of rows in the matrix, x<sub>ii</sub> is the number of observations in row i and column i, x<sub>i+</sub> and x<sub>+i</sub> are the marginal totals of row i and column i, respectively (Bishop et al. 1975).</p>
<b>Commission error</b>	Show false positives or overestimation; occurs when a classification procedure assigns pixels to a certain class that in fact don’t belong to it.
<b>Omission error</b>	Show false negatives or underestimation; occurs when pixels that in fact belong to one class, are included into other classes.

### 3.2.2.5 Mussel Coverage and abundance

In order to determine mussel coverage, we divided the classified image in three equally sized sections. In each sample, we calculated the real (not projected) area for the “mussels” class and “rocky” and “algae” substrate classes, taking into account the 3D topography of the terrain. Real areas were calculated from the Digital Elevation Models using the SAGA 2.1.2 algorithm (Conrad et al. 2015). Geoprocesses involved in the combination of the classified images and the DEM were carried out in QGIS 2.14 (QGIS Development Team 2016). The ratio of mussel coverage per  $m^2$  of available rocky substrate was then calculated. In order to estimate the total number of mussels in the image, we used the ground quadrat samples (20cm x 20cm) as a reference (Fig. 3.3); the total number of mussels in each quadrat was multiplied by the quadrat mussel coverage to determine the number of mussels per  $m^2$  of rocky substrate (individuals/ $m^2$ ) and the average density of the ground quadrats of each image was used to transform mussel coverage to density in the rest of the image.

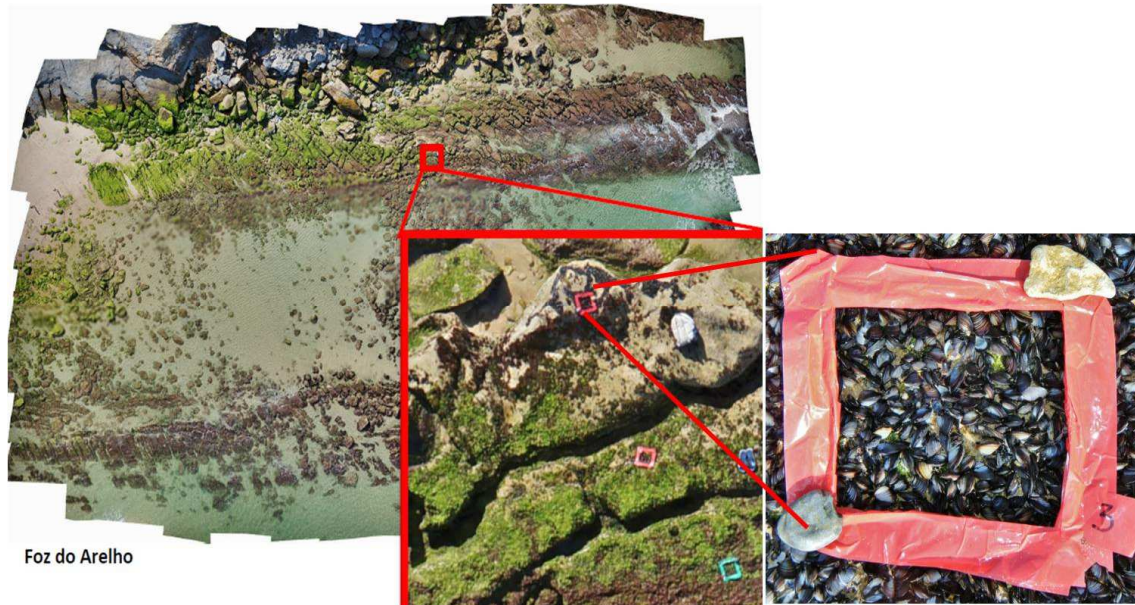


Figure 3.3 Example of an orthophoto mosaic, a zoomed view and a ground image of the field quadrat (from left to right). The red quadrats included mussels and the green quadrats include any other feature (rocks, algae, shadows). These quadrats were used to assist in the accuracy assessment analysis.

### 3.2.3 Environmental variables

#### 3.2.3.1 Wave Fetch

We calculated wave fetch using a grid map (grids of 200 m distance cells) of the coastline and extending to 200 km on the sea side, using the methodology described in Burrows et al. 2008 which considered 200 km as the maximum fetch distance to influence wave conditions. For each coastal cell, we defined 16 equal angular sectors ( $22.5^\circ$ ) and the wave fetch was determined as the distance (in km) to the closest land in each angular sector (from 0, where the vector reach land, to 200 when the vector reached the open sea at the maximum distance). After calculating the sum of all sector distances for each coastal cell ( $F_{sum}$ ), we averaged that value with the two immediately neighbouring cells ( $F_{3avg}$ ). This was done to create more representative values of the coastline at a local scale, given the coastline conversion from a line to a 200m resolution grid.

#### 3.2.3.2 Wind Energy

Hourly wind data (direction and speed) was provided by IPMA (Instituto Português do Mar e da Atmosfera) for 5 weather stations: Cabo Carvoeiro, Praia da Rainha and Setúbal (from January 2004 to December 2014), Santa Cruz (from April 2012 to December 2013) and Cabo Raso (from February 2004 to December 2014). Using QGIS 2.14, we then matched each coastal cell with the closest weather station (Fig. 3.1) to calculate wind energy. In each cell, we calculated wind energy as the average from the product of the proportion of time the wind blew in each of the 16 sectors by the square of the average wind speed, in knots. We divided the year in two main seasons: summer (from April to September) and winter (from October to March).

#### 3.2.3.3 Wave Exposure Index (WEI)

The Wave Exposure Index was determined in every coastal cell as the average of the products of the wave fetch and wind energy for each of the 16 sectors, for the summer and winter season separately.

#### 3.2.3.4. Terrain Roughness Index (TRI)

Terrain Roughness Index was calculated as the sum of the absolute change in elevation between a grid cell and the neighbour cells around a 20 cm<sup>2</sup> area, using SAGA 2.1.2 algorithms.

### 3.2.4 Spatial Extrapolation

Generalized additive models (GAMs), as implemented in the *mgcv* library of R 3.3.2 (R Development Core Team 2016), were used to investigate the effect of the environmental variables on mussel size (L), and density (individuals/m<sup>2</sup> rocky intertidal). In order to accomplish normality and homoscedasticity, mussel size and density were log-transformed. Wave Exposure Index and Terrain Roughness Index were included as smoothed terms in the models and estimated with thin plate regression splines. The large correlation detected between the summer and winter WEI ( $r=0.85$ ) prevented the use of both variables together in the same model. The possible effect of the shoreline type (Boulders or Flat Platform) was also included in the model as a factor. Model selection was based on the Akaike Information Criterion (AIC) and model validation of the selected models included the verification of homogeneity (lack of structure of the residuals) and normality (quantile–quantile plot of the residuals).

Selected models were then used to extrapolate mussel density and size along the central Portuguese west coast. Reproductive output (RO, N<sup>o</sup> eggs/m<sup>2</sup>) was calculated for each location, using the estimates of population density, average body-size and the known relationship between egg production and mussels length ( $\log(\text{N}^{\circ}\text{Eggs/mussel}) = -0.133 + 2.304 * \log\text{Size}$ ;  $R^2=0.3$ ; Siregar, 2014).

## 3.3 Results

### 3.3.1 Image processing and classification

The weather condition during the survey week was similar for all locations, with good visibility and low winds. However, the image acquisition flights were planned to occur during very low tides, which coincided in some days with very early mornings or late afternoons. This way, Porto Novo and Peniche images contained a large amount of shadow and diminished brightness. Other images presented other challenges due to topography, especially in the dynamic area where waves meet the shore causing image mismatching and errors in some areas (e.g. Foz Arelho and Galapos). Individual image collection at the different survey sites ranged from 82 (Maçãs) to 247 (Porto Novo). The resultant survey orthophoto mosaics had an image overlap error of less than 1 pixel for all locations, and covered an average area of 15200 m<sup>2</sup> (with a minimum of 5900 m<sup>2</sup> in Maçãs and a maximum of 25900 m<sup>2</sup> in Porto Novo) with a ground resolution of approximately 0.8 cm/pixel. Total camera location error (m) regarding the camera GPS and the Agisoft's model prediction was around 1.5 m for all locations, except Foz Arelho where it reached 6.34 m (Table 3.2).

Table 3.2 - Survey data and camera location error.

	Survey data					Average camera location error			
	Number of images	Flying altitude (m)	Ground resolution (cm/pix)	Coverage area (m <sup>2</sup> )	Image overlap Error (pix)	X error (m)	Y error (m)	Z error (m)	Total error (m)
<b>Foz Arelho</b>	131	31.44	0.84	16600	0.99	5.80	2.49	0.65	6.34
<b>Peniche</b>	132	32.80	0.87	15400	0.87	2.25	1.97	0.38	3.02
<b>Porto Novo</b>	247	33.11	0.89	25900	0.93	1.28	0.53	0.69	1.55
<b>Samarra</b>	116	27.23	0.73	13200	0.78	1.08	1.42	0.81	1.97
<b>Maças</b>	82	29.36	0.77	5900	0.57	0.42	1.27	0.81	1.57
<b>Mexilhoeira</b>	137	31.09	0.84	14000	0.76	0.76	0.95	0.38	1.28
<b>Bafureira</b>	90	31.66	0.86	14000	0.82	0.52	0.64	0.35	0.90
<b>Galapos</b>	108	30.17	0.80	16400	0.92	1.00	0.36	0.39	1.14

For each aerial survey, we obtained four main outputs: an orthophoto mosaic, a classified image based on the ground-cover types, a Digital Elevation Model and a Terrain Roughness Index raster image (Figure 3.4 and Supplementary information 1).

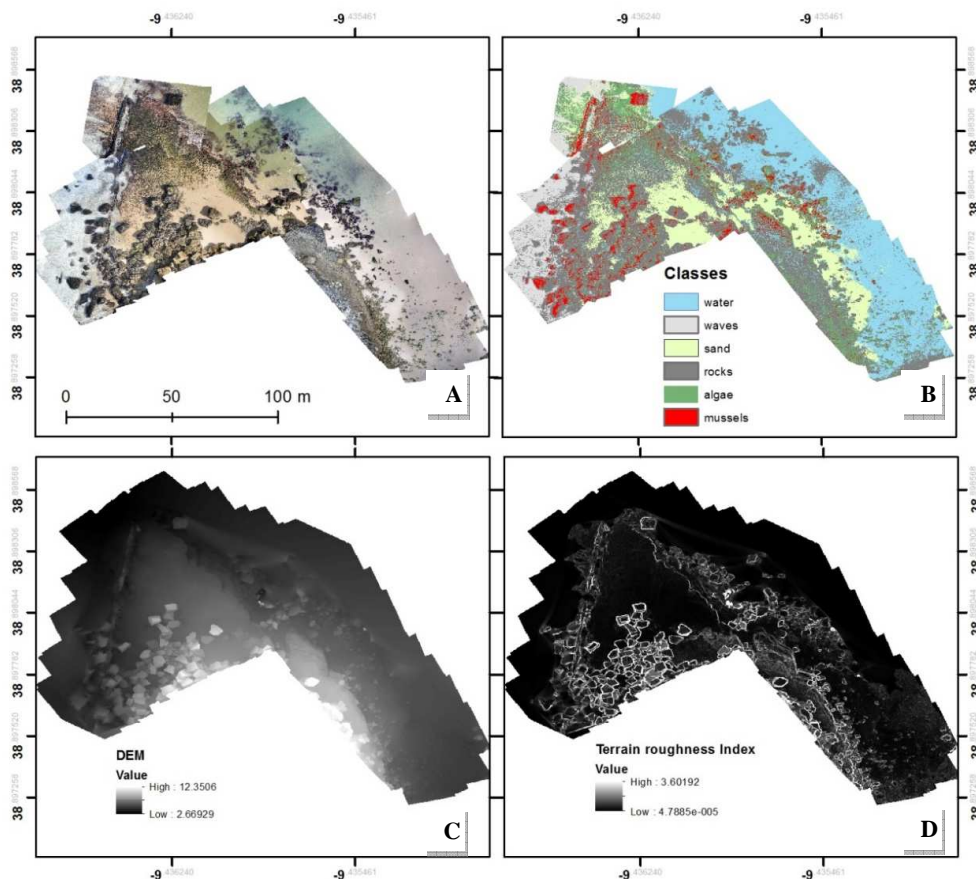


Figure 3.4 **A** - Orthophotomosaic of Samarra intertidal rocky shore derived from 33m altitude drone flight. **B** – Classified image (supervised image classification) showing the membership of the pixels in the mosaic to each class. **C** – Digital Elevation model (DEM, in meters). **D** – Terrain roughness index.

Classified images accuracy was assessed through error matrices of pixel membership in the selected classes (table 3.3). Overall accuracy and kappa coefficient were generally high for all images:  $86.4 \pm 4.3\%$  and  $83.1 \pm 5.1\%$  (mean $\pm$ SD), respectively. However, different ground-cover classes diverged in classification accuracy (see supplementary information 2 for the full set of error matrices). In general, the more homogeneous classes of water and sand were more accurately classified when compared to the classes of algae, rocks and mussels. Regarding the mussel class cover, commission errors (or overestimation) were higher in Porto Novo (32.7%) and Maçãs (30.1%), while omission errors (or underestimation) prevailed in Foz Arelho (27.6%) and Porto Novo (26.7%). Galapos and Mexilhoeira classified images showed the highest overall accuracy and the smallest classification errors for the mussel class (table 3.3).

Table 3.3 Error matrix expressing the accuracy assessment of pixel membership in the overall image and for each individual class. For the purpose of this work, we only show the mussel class accuracy results.

<b>Classified Images</b>	<b>Overall</b>		<b>Mussel class cover</b>	
	<i>Overall accuracy (%)</i>	<i>Kappa coefficient (%)</i>	<i>Commission error (%)</i>	<i>Omission error (%)</i>
Foz Arelho	82.2	79.7	8.9	27.6
Peniche	83.2	78.8	0.1	13.7
Porto Novo	80.8	75.8	32.7	26.7
Samarra	86.1	82.7	12.5	13.4
Maçãs	87.6	83.2	30.1	7.3
Mexilhoeira	88.6	86.1	0.2	5.6
Bafureira	89.2	86.9	2.3	20.3
Galapos	94.1	92.1	0.3	5.0

Using the classified images, and the DEM, we calculated the 3D surface area for mussel and rocky substrate cover, for each image. An example of the orthophoto, classified image and DEM is given in Figure 3.4 (for other location, see supplementary information 1). Mussel coverage (ratio mussels/rocky substrate) was highest in Maçãs ( $\approx 60\%$ ), followed by Porto Novo ( $\approx 20\%$ ), Samarra ( $\approx 16\%$ ), Mexilhoeira ( $\approx 9.3\%$ ), Galapos ( $\approx 6.1\%$ ), Foz Arelho ( $\approx 5\%$ ), Bafureira ( $\approx 4.5\%$ ) and Peniche (0.4%). As for mussel density per rocky substrate area, Maçãs also showed the highest density ( $\approx 6500$  mussels/m<sup>2</sup>), and Peniche the lowest ( $\approx 58$  mussels/m<sup>2</sup>). These two locations also presented the smallest mussels (an average length of 1.9 and 1.6 cm in Maçãs and Peniche, respectively) while in Porto Novo the average mussel size reached 3.8 cm (Table 3.3).

### 3.3.2 Environmental variables

Wave Fetch and Wave Exposure Indices estimated for the study area are shown in Figure 3.5.

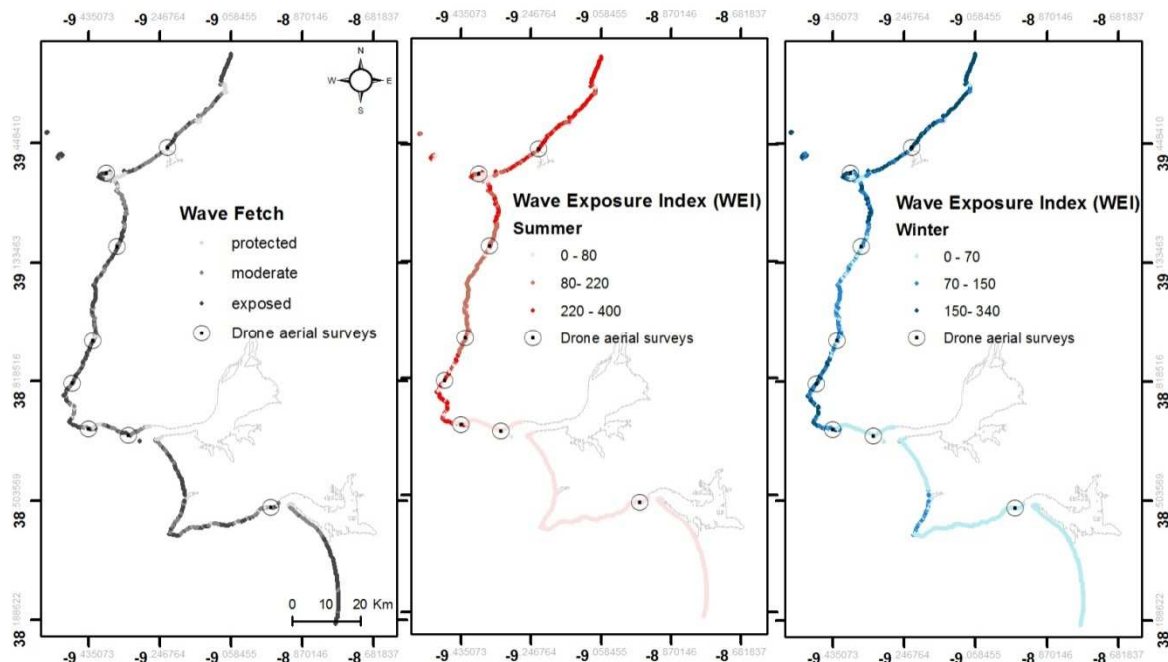


Figure 3.5 Wave Fetch (represented by three primary categories for the coastal cells: protected <500, moderate 500-1000 and exposed >1000) and Wave Exposure Index (WEI) for summer and winter seasons. Round circles illustrate drone aerial survey locations: from North to South: Foz Arelho, Peniche, Porto Novo, Samarra, Maçãs, Mexilhoeira, Bafureira and Galapos.

Wave fetch differed amongst survey sites, from more exposed sites (Peniche, Foz do Arelho, Maçãs) to more protected locations (Galapos, Samarra, Bafureira) (Figure 3.5, Table 3.4). The wind information (wind velocity (m/s) and frequency (%) by coastal sector) for the 5 weather stations (Fig. 3.6) showed patterns consistent with a typical upwelling environment, with stronger northern and northwesterly winds during the summer, and higher values in the weather stations located in the exposed coastline (Cabo Carvoeiro, Santa Cruz and Cabo Raso).



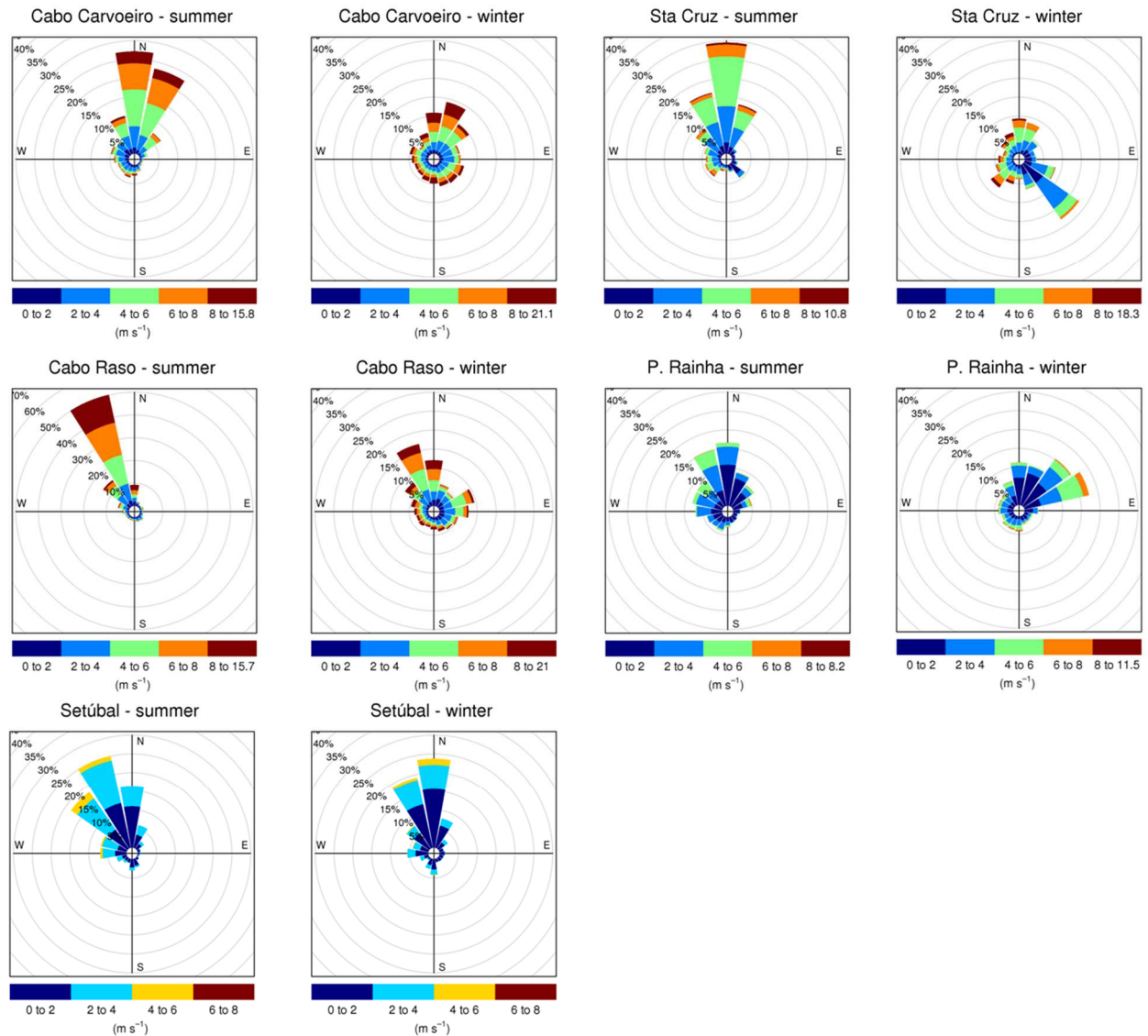


Figure 3.6 Wind patterns (wind velocity ( $\text{m/s}$ ) and frequency (%) by coastal sector) for the 5 weather stations and divided in two main seasons: summer (from April to October) and winter (from October to March).

The Wave Exposure Index (WEI) in the survey locations showed highest indexes in the summer when compared to the winter season. Galapos had the lowest WEI in both seasons, while Maçãs and Mexilhoeira showed the highest WEI for summer and Peniche and Foz Arelho evidenced higher winter WEI. Finally, regarding Terrain Roughness Index (derived from the digital elevation model), Maçãs showed the lowest index (0.028) and Porto Novo the highest (0.16). Table 3.4 summarizes image mussel coverage, density and size, and all environmental estimates for each of the survey locations.

### 3.3.3 Generalized additive models

No significant effects of shore morphotypes (boulders or flat platform) or Terrain Roughness Index (TRI) on mussel density were detected. Regarding the environmental variables, only the effect of winter WEI was significant, explaining 47.2% of the variability observed on mussel density (Table 3.5; Figure 3.5A). Maximum mussel density occurred at intermediate values of this index (Figure 3.7A).

Concerning size, the model including just winter WEI explained more than 40% of the variability (43.2%) showing a progressive decrease in size as Winter WEI indexes exceeded intermediate values (Figure 3.7B). Including TRI in the model amplified the variability explained to 57.3% and reduced the Akaike Information Criterion (AIC) (Table 3.5). The effect of winter WEI kept the same relationship with an optimum of density at intermediate values (Figure 3.7C) but this model revealed a linear (edf=1; Table 3.5) and positive relationship between size and the heterogeneity of the rocky substrate (Figure 3.7D). Nonetheless, shore morphotype (boulders and flat platforms) did not show a significant effect on mussel size ( $p > 0.05$ ) for any of the model combinations tested. In brief, we found higher mussel density at intermediate levels of WEI exposure in winter, smaller mussels at high WEI and bigger mussels where substrate complexity was higher.

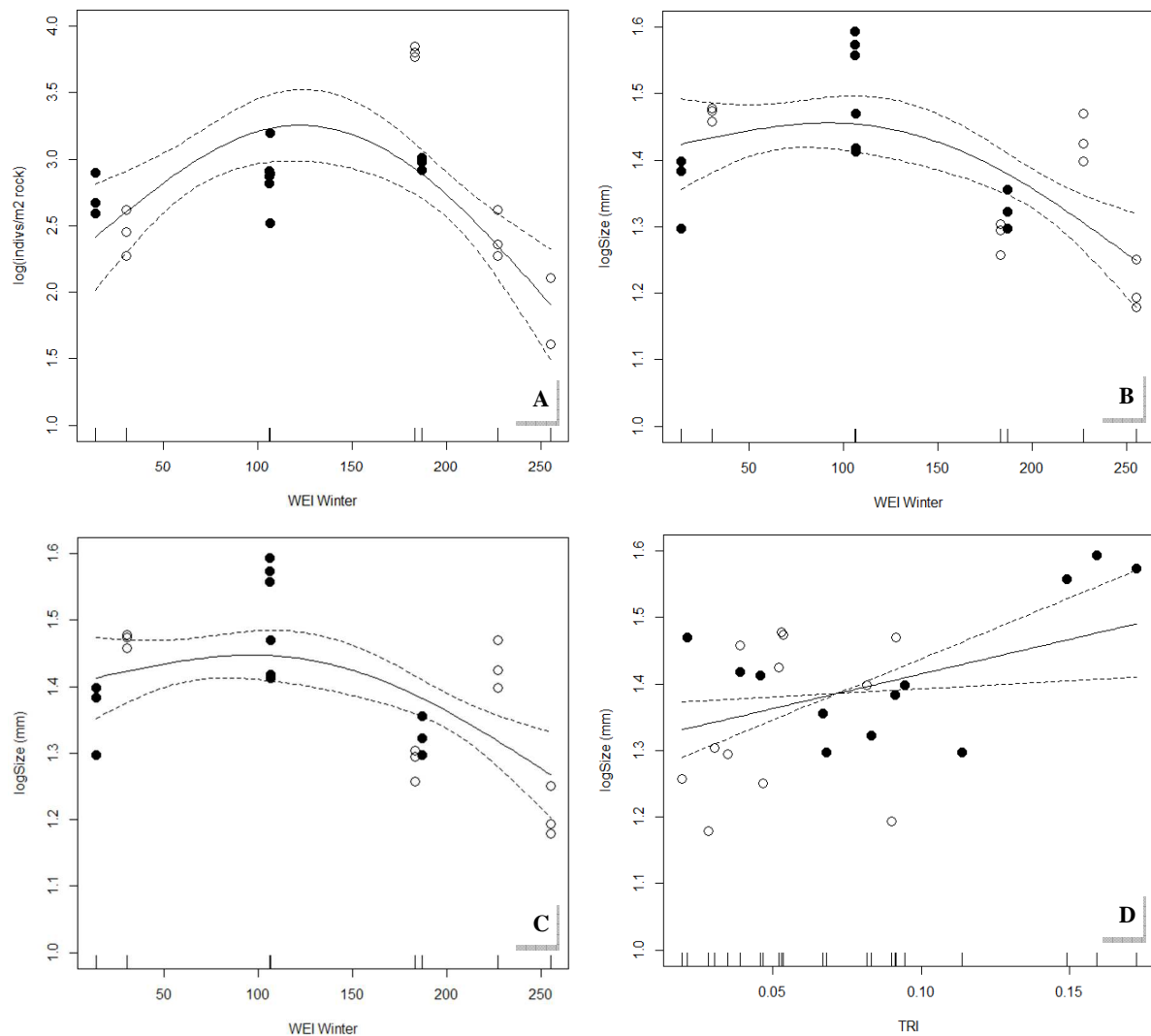


Figure 3.7 Generalized additive models results showing the effect of winter WEI on mussel density ( $\log(n^\circ \text{ mussels}/\text{m}^2)$ ) **(A)** on mussel size ( $\log(\text{Size (mm)})$ ) **(B)** as well as the partial effect of winter WEI **(C)** and TRI **(D)** on mussel size for the model which combines both variables (Table 3.3). Dotted lines indicate 95% confidence intervals, and tick marks along the x-axis below each curve represent effect values where observations occurred. Open circles indicate flat platform morphotype while filled circles represent boulder shores.

Although including TRI in the model increased the accuracy to predict mussel size, we used the models which only accounted for the winter WEI effect (Figure 3.7A) to extrapolate our results to the rest of the central Portuguese west coast, since TRIs were not available for the whole study area. Figure 3.8 illustrates the *M. galloprovincialis* distribution maps regarding mussel's abundance, size and reproductive output along the rocky coast in the study area, based on model predictions. The maps highlight areas with low to intermediate winter WEI as the most

relevant from the reproductive output point of view, because of the combination of higher mussel density and larger individuals.

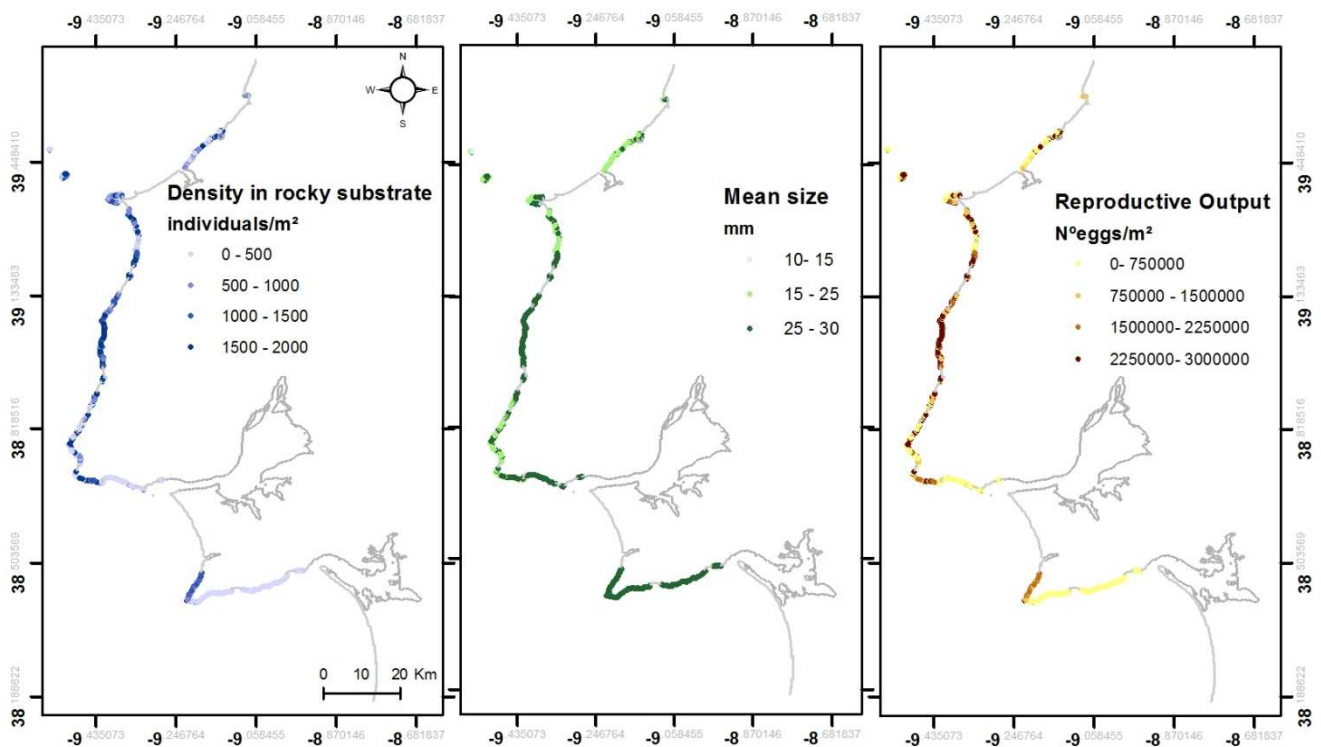


Figure 3.8 *Mytilus galloprovincialis* predicted density, mean size and reproductive output in the central west Portuguese rocky shore.

Table 3.4 – Drone aerial survey image estimates for mussel and rocky substrate cover, density and size, and environmental variables used in the GAMs. \* represents image sections

Drone aerial surveys	*	Image processing						Environmental variables							
		Mussel cover 3D (m²)	Rocky substrate cover 3D (m²)	Ratio mussel: rocky substrate	Average mussel density in field quadrats (indiv/m²)	Average mussel density on the shore (indiv/m²)	Average mussel size in field quadrats (cm)	Wave Fetch (F3avg)	Weather Station	Wind Energy Summer	Wind Energy Winter	Wave Exposure Index Summer	Wave Exposure Index Winter	Mean Terrain Roughness Index	Shoreline type
Foz Arelho	1	120.677	2749.392	0.044	5220.429	229.136	2.660	1007.3	Cabo Carvoeiro	26.756	35.688	274.994	227.170	0.052	flat rocky platform
	2	238.095	2994.176	0.080	5220.429	415.125	2.950							0.091	flat rocky platform
	3	96.768	2702.812	0.036	5220.429	186.906	2.500							0.082	flat rocky platform
Peniche	1	437.674	47571.302	0.009	13928.953	128.152	1.560	1137.4	Cabo Carvoeiro	26.756	35.688	248.548	254.949	0.090	flat rocky platform
	2	140.249	48107.451	0.003	13928.953	40.607	1.510							0.028	flat rocky platform
	3	16.648	48660.593	0.000	13928.953	4.765	1.780							0.047	flat rocky platform
Porto Novo	1	1125.126	5173.560	0.217	3698.778	804.396	3.750	1003.5	Stcruz	17.634	19.966	166.125	106.101	0.172	boulder fields
	2	660.564	3292.824	0.201	3698.778	742.001	3.920							0.159	boulder fields
	3	582.010	3225.787	0.180	3698.778	667.349	3.610							0.149	boulder fields
Samarra	1	275.274	4647.534	0.059	5580.751	330.548	2.620	813.6	Stcruz	17.634	19.966	166.268	106.868	0.039	boulder fields
	2	391.484	1380.050	0.284	5580.751	1583.114	2.590							0.046	boulder fields
	3	122.425	889.225	0.138	5580.751	768.335	2.950							0.021	boulder fields
Maças	1	826.432	1166.844	0.708	10054.968	7121.560	2.010	1005.6	Cabo Raso	34.342	30.308	338.700	183.359	0.030	flat rocky platform
	2	561.587	900.172	0.624	10054.968	6272.956	1.810							0.019	flat rocky platform
	3	529.496	897.788	0.590	10054.968	5930.199	1.970							0.035	flat rocky platform
Mexilhoeira	1	230.530	2440.719	0.094	10032.552	947.593	2.100	938.8	Cabo Raso	34.342	30.308	355.741	186.767	0.083	boulder fields
	2	267.248	3225.587	0.083	10032.552	831.224	2.270							0.067	boulder fields
	3	418.775	4126.865	0.101	10032.552	1018.055	1.980							0.068	boulder fields
Bafureira	1	117.174	2729.946	0.043	6603.640	283.439	2.980	933.3	P.Rainha	7.177	9.745	59.771	30.624	0.054	flat rocky platform
	2	99.125	3500.263	0.028	6603.640	187.010	2.870							0.039	flat rocky platform
	3	183.525	2917.836	0.063	6603.640	415.353	3.010							0.053	flat rocky platform
Galapos	1	113.520	2646.096	0.043	9119.045	391.214	2.420	766.5	Setúbal	5.519	3.715	36.838	14.141	0.091	boulder fields
	2	155.264	3054.052	0.051	9119.045	463.599	2.500							0.095	boulder fields
	3	259.664	2957.599	0.088	9119.045	800.611	1.980							0.114	boulder fields

Table 3.5 - Structure of the significant General Additive Models detected to describe the effects of the environmental and morphometric variables on mussel density (A) and Size (B). edf: estimated degrees of freedom for the smooth terms. AIC: Akaike Information Criterion. Values in bold indicate statistical significance.

<i>Variable</i>	Parametric Coefficient					Smooth terms (non parametrics)						
	Parameter	Estimate	S.E.	t	P	Parameter	e.d.f	F	P	R <sup>2</sup> adj.	% Deviance explained	AIC
<b>A)</b>												
<i>log(Density)</i>	Intercept	2.695	0.104	25.69	<b>&lt;2x10<sup>-16</sup></b>	Winter WEI	1.94	8.706	<b>0.001</b>	0.423	47.20%	
<b>B)</b>												
<i>log(Size)</i>	Intercept	1.386	0.018	75.9	<b>&lt;2x10<sup>-16</sup></b>	Winter WEI	1.85	6.851	<b>0.004</b>	0.382	43.20%	-43.10
<i>log(Size)</i>	Intercept	1.386	0.016	85.44	<b>&lt;2x10<sup>-16</sup></b>	Winter WEI	1.85	6.109	<b>0.006</b>	0.513	57.30%	-47.94
						TRI	1	6.822	<b>0.016</b>			

### 3.4 Discussion

#### 3.4.1 Drone aerial survey estimates

The results of this study indicate that high-resolution images obtained from drone platforms, together with the quantification of fine-scale geomorphologic substrate attributes, show great potential for intertidal monitoring protocols at an ecologically relevant scale. The drone's ability to be quickly and repeatedly deployed offer the possibility to quantify spatial patterns of intertidal rocky organisms and vegetation otherwise not detectable with satellite imagery or piloted aircrafts. Recognizing these patterns is the first step to understand how species distribution is shaped by the environment, and develop broad scale metapopulation models to increase ecosystem sustainability (Rango et al 2006).

The use of the maximum likelihood classification algorithm produced very high-resolution ground-cover classification maps useful for general spatial and temporal identification and characterization of landscape and functional groups diversity in rocky intertidal areas. However, in pixel-based image analysis the main challenge has to do with the determination of a RGB band spectrum which is representative and distinctive for the ground-class training set (Foody et al 2004, 2006). The quality of the pixel-range training set for each class depends on image resolution, the features of interest to be categorized, the natural light, amount of shadow present and the image analyst expertise of the landscape. Ideally, areas used as training sites should derive from ground data, and be accessible for ground truthing and verification. Since both training sets and validation samples for accuracy assessment are derived from visual interpretation of the same image, some subjectivity is inherent in the overall image analysis. This has been a limitation in achieving accurate automatic classification of complex landscape from low resolution remote-sensing imagery (Manandhar, Odeh, and Ancev, 2009). However, were able to use the ground quadrats mussel pixels to test accuracy of the mussel class, and working with high resolution images ( $\approx 1 \text{ cm}^2$ ), such as the ones used in this study, help to identify clear and unambiguous features on the image and avoid scene-dependent errors.

Yet, due to absence of near infrared imagery, which is more suitable for vegetation classification, we kept a general class for vegetation, since our goal was to develop a protocol capable of separating the mussel class cover type from the rocky/algae substrate. Importantly, our resulting maps can still easily recognize the size, patchiness and meadow boundaries of vegetated intertidal areas, highlighting other potential uses of these techniques.

In our study, class cover misclassification errors involving the mussel class were caused mostly by spectral confusion between the mussel class and classes of algae or rocks in the shadow. These errors in the classified maps take place when classes are not spectrally separable or atmospheric effects mask fine differences. This illustrates some difficulty in separating land-cover classes based on spectral signatures, especially given the low contrast in vegetation cover and associated biota accentuated by shadow conditions in 3D complex environments. At a species level classification, mixed-pixel effects can restrict the use of aerial imagery in detecting fine-scale processes. In our case, it is possible that the mussel shells were not easily distinguished from dark-colored rocks, especially in shadow areas and areas exhibiting dark encrusted algae growing on the rocks. Object-based image analysis, rather than pixel-based have shown to perform better classifications with high and very high-resolution images exhibiting low spectral information or shadow (Yu et al. 2006).

The contemporary development in image acquisition and processing techniques dealing with multispectral sensors (Zeng et al. 2017), image segmentation parameters (Johnson and Jozdani 2018), texture and object-based classifications (Laliberte and Rango 2009) and shadow effects (Milas et al. 2017) will likely improve spectral differentiation and ground cover class differentiation in the future. Moreover, fine-scale digital surface models hold great promise for a number of applications in ecological studies.

Konar and Iken 2017, in rocky intertidal strata within a seagrass bed, compared images captured by drone with data derived from observers on the ground. As expected, the authors found that the observer data achieved higher resolution of taxonomic categories, but on the coarse taxonomic resolution drone imagery could detect larger spatial scale (regional) and differences in overall community structure. Importantly, Murfitt et al. 2017 showed that the total time taken to complete the drone survey of the intertidal reef was half the time of on-ground quadrat observations, with no significant differences between drone and on-ground estimates for a dominant single-species canopy cover. Duffy et al. 2018, using very fine spatial resolution aerial images were able to identify meadow features such as lugworm (*Arenicola sp.*) mounds and cockle shells (*Cerastoderma edule*).

Our study, integrating low altitude aerial images and ground surveys provided overall high accuracy classification images and allowed the quantification of mussel coverage and density along heterogeneous stretches of intertidal rocky shores. Moreover, the fact that drone imagery produced 3D high resolution orthogonal models proved useful to measure habitat complexity and calculate realistic surface coverage in highly heterogeneous rocky substrates.



### 3.4.2 Model estimates

Regarding the analysed environmental variables, our results highlight the relevance of wave exposure on the density and distribution of mussel's populations along the Portuguese coast. Although winter and summer WEIs were highly correlated ( $r=0.85$ ), only winter WEI had a significant effect on mussel abundance, pointing out the stronger hydrodynamic stress suffered during winter. However, WEI indexes reached higher values during summer for most locations, which can be seen in Figure 3.5. This pattern of large values of WEI observed during summer are driven by stronger N and NW upwelling predominant winds characteristic of this season. Still, wave energy has been described as much higher during fall-winter than spring-summer (Silva et al. 2012; Ramos et al. 2017). Wave seasonal patterns in the Iberian Peninsula are highly dependent on the North Atlantic Oscillation index (NAO) which represents differences of atmospheric pressure between the Iceland Low and the Azores High systems. Characteristic positive values of NAO during winter-fall represent high pressure differences between these two systems and are associated to stronger storms, larger wave heights and a predominant North-Eastward wave direction which is also associated to larger alongshore sediment transport (Silva et al. 2012; Ramos et al. 2017). This way, on the Portuguese west coast, although the wave regime is dominated by swells from the NW, storm periods with extreme wave conditions are associated with prevailing southerly winds and downwelling conditions (Vitorino et al. 2002), occurring during the winter months with high wave heights (Pita and Santos, 1989). This way, adding wave energy data (wave direction and significant height) from local oceanographic buoys would greatly improve our understanding the winter wave regime in our study way.

Our results indicate that WEI is only determinant for mussel densities during the periods when wave energy is high enough to cause dislodgement stress.

Wave generated hydrodynamic stress strongly influences intertidal communities (e.g. Paine and Levin 1981, Denny and Wetthey 2001, Lindsay and McQuaid, 2007), affecting species vertical distribution and their trophic relationships (McQuaid and Branch 1985). Some nearshore areas in Portugal have been characterized of considerably high energy, given the country's location at relatively high latitude, orientation and the presence of a stretched area of ocean immediately to the west. High energy waves were characterized in the extreme north of the Portuguese coastal environment and in the central region around Peniche and Cabo da Roca areas (Rusu and Soares 2009; Ramos et al. 2017).

Water motion influences fertilization (Pearson and Brawley 1996), larval input and settlement (Hunt and Scheibling 1996), growth (McQuaid and Lindsay 2000), nutrient and sediment cycling [Griffiths and Hammond, 2004], species dislodgement (Rius and McQuoid 2006),

species competitive interaction (Branch and Steffani 2003) and even shell morphology (Stewart and Carpenter 2003). This way, as wave action increases, nutrient and larval cycling also increases, along with amplified hydrodynamic stress, critical for the attachment, growth and survival of sessile intertidal communities. In this manner, wave action is a limiting factor for intertidal organisms' growth and survival, forcing them to adapt, withstand and cope with the constant dynamics of water motion (Carrington et al. 2008).

Our results indicate maximum mussel densities at intermediate wave exposure values during winter in agreement with several studies demonstrating that *M. galloprovincialis* is present in a wide range of sheltered and exposed conditions, but thrive under intermediate degrees of wave exposure with highest abundance.

Exposed sites with intermediate levels of wave exposure seem to favour mussel relative abundance (Willis and Skibinski 1992, CáceresMartínez et al. 1993, van Erkom Schurink and Griffiths 1993, Bustamante and Branch 1996a, Bustamante et al. 1997, Branch and Steffani 2003, 2004). The rationale behind this trend might be that high water flow increases food delivery and larval supply, as well as limiting predation, thus promoting faster growth and larger densities; however, high water flow and wave impact may also increase dislodgement (e.g. Menge 1978, Denny 1988, Bertness et al. 1991, van Erkom Schurink and Griffiths 1993, Dahlhoff and Menge 1996). This way, important limiting factors seem to play critical roles at either end of the wave exposure range: sheltered sites having diminished larval input and nutrient cycling with fewer settlement, slower growth and increased benthic predation (Menge 1976) and very exposed sites causing too much physical stress, diminished opportunities for attachment and greater mussel dislodgment (Seed and Suchanek 1992). This trend has been reported for several mytilid species in different biogeographic locations. Steffani and Branch (2003), in the West Coast of South Africa, found greatest recruitment and growth rates for *M. galloprovincialis* at exposed sites in comparison to sites sheltered from direct wave action or sites exposed to extreme wave action. Blanchette et al. (2007), in California, also found that *M. galloprovincialis* grew faster at moderately exposed sites when compared to sheltered and extremely exposed sites and Westerborn and Jattu 2006, in the Baltic Sea evoked higher *Mytilus edulis* biomass at areas with intermediate wave exposure. In northwest Atlantic rocky intertidal habitats the distribution of *Mytilus edulis* and *M. trossulus* along a full gradient of wave exposure yielded similar results: low density in very sheltered and very exposed habitats, and high density in intermediate exposed habitats (Tam and Scrosati 2014). In addition, in a broad scale general description of rocky shore distribution patterns along the whole Portuguese coast, *M. galloprovincialis* was present along the entire coast but showed a progressively decline in abundance from north to south, following a general

decrease in exposure (Boaventura et al 2002). Similarly, in a recent study of the SW Portuguese coast, the abundance of *P. pollicipes* was modelled as a function of a fetch-based exposure indices and the barnacle abundance increased non-linearly with fetch.

Regarding mussel's size, we found larger mussels at low and intermediate values of wave exposure. In South Africa, larger individuals of the invasive *M. galloprovincialis* are also found at intermediate levels of shore exposure (McQuaid et al. 2000; Hammond and Griffiths, 2004). O'Connor 2010 reported less dense and larger mussels on sheltered rocky shores on Atlantic coasts in Ireland and other studies also reported smaller mussels in more exposed shores (e.g. Alvarado and Castilla, 1996). For high wave energy environments, Hunt and Scheibling (2001) described a greater probability of dislodgment for larger mussels than smaller ones. Additionally, by using 3D profile irregularity data to characterize topographic complexity, our study found a relation between mussel size and terrain roughness index, with bigger mussels appearing in highly heterogeneous rocky substrates. This indicates that irregularity seems to favour growth and provide shelter from physical stress, allowing larger mussels to survive. In aquatic ecosystems, the importance of three-dimensional structures in providing protected habitat space to organisms is well recognized (Tokeshi, 1999; Bruno and Bertness, 2001; Kawai and Tokeshi, 2004).

Estimations of mussel size are especially relevant to calculate reproductive output of different populations, and their contribution to the persistence of the meta-population. Although fertility can be influenced for many factors (temperature, food availability, parasites, etc.) gonad size is determinant and mussel length has been documented to explain as much as 30% of the variability observed in egg production (Siregar, 2014). This way, simple and easy to obtain variables at long spatial scales, can assist in identifying hotspots for reproduction and survival.

Nonetheless, we should notice that several factors, other than wave exposure, are likely to explain mussel density, size and reproductive output in the study area. At a smaller spatial scale, biological interactions within the intertidal community (competition and predation) or the type of substrate and topography, such as slope and inclination can shape mytilid distribution. On a larger spatial scale, temperature and dissection effects, heterogeneous harvesting pressure and variation in the intensity of coastal upwelling can play an important role in the recruitment and survival of benthic invertebrates along intertidal shores. Also, episodic storm events have been shown to strongly affect mortality of mussels in exposed areas (Zardi et al. 2006).

This study showed the importance of wave exposure in shaping mussel densities in rocky substrates and consequently, reproductive output. These results are crucial given that climate

change is increasing levels of wave action globally (Young et al. 2011) and increasing storms and wave height in the Atlantic (e.g. Andrade et al 2007) with potential increase in the environmental stress upon the coastal ecosystems and activity. Understanding and predicting patterns of distribution and abundance of key ecosystem engineers is important to generate ecological models of broad scope and validity for coastal ecosystems conservation purposes. Studies such as this one provide comprehensive protocols to assist data-acquisition techniques and methodologies which can be used on multiple spatial and temporal scales, to effectively identify habitats and species distribution patterns in rocky intertidal areas.

### 3.5 Conclusions

In this study we have confirmed the potential of drone-based aerial surveys and image techniques for monitoring intertidal rocky shores. In brief, combining automated images classification methodologies for intertidal species distribution with fine-scale topographic GIS data integration and environmental modelling proved to be a useful way to get large scale information along heterogeneous and dynamic stretches of intertidal rocky shores.

The protocol developed here provided a time cost efficient protocol to assist foundational research questions in ecology, such as dealing with distribution patterns of organisms and the way they interact with the biotic and abiotic environment. Our predictive model of mussel abundance, size, and reproductive output, was built to meet practical management and forecasting needs. However, further research work, such as additional drone aerial surveys are needed to further validate its results, and reflect on whether the model mimics the real world well enough for its purpose. Reproductive output has been described as key information to understand the persistence of spatially-structured populations within heterogeneous and patchy habitats (e.g Trembl et al 2012, Burgese et al 2014). This way, our location-specific predictions can be used in metapopulation models dealing with the management of pivotal conservation areas.

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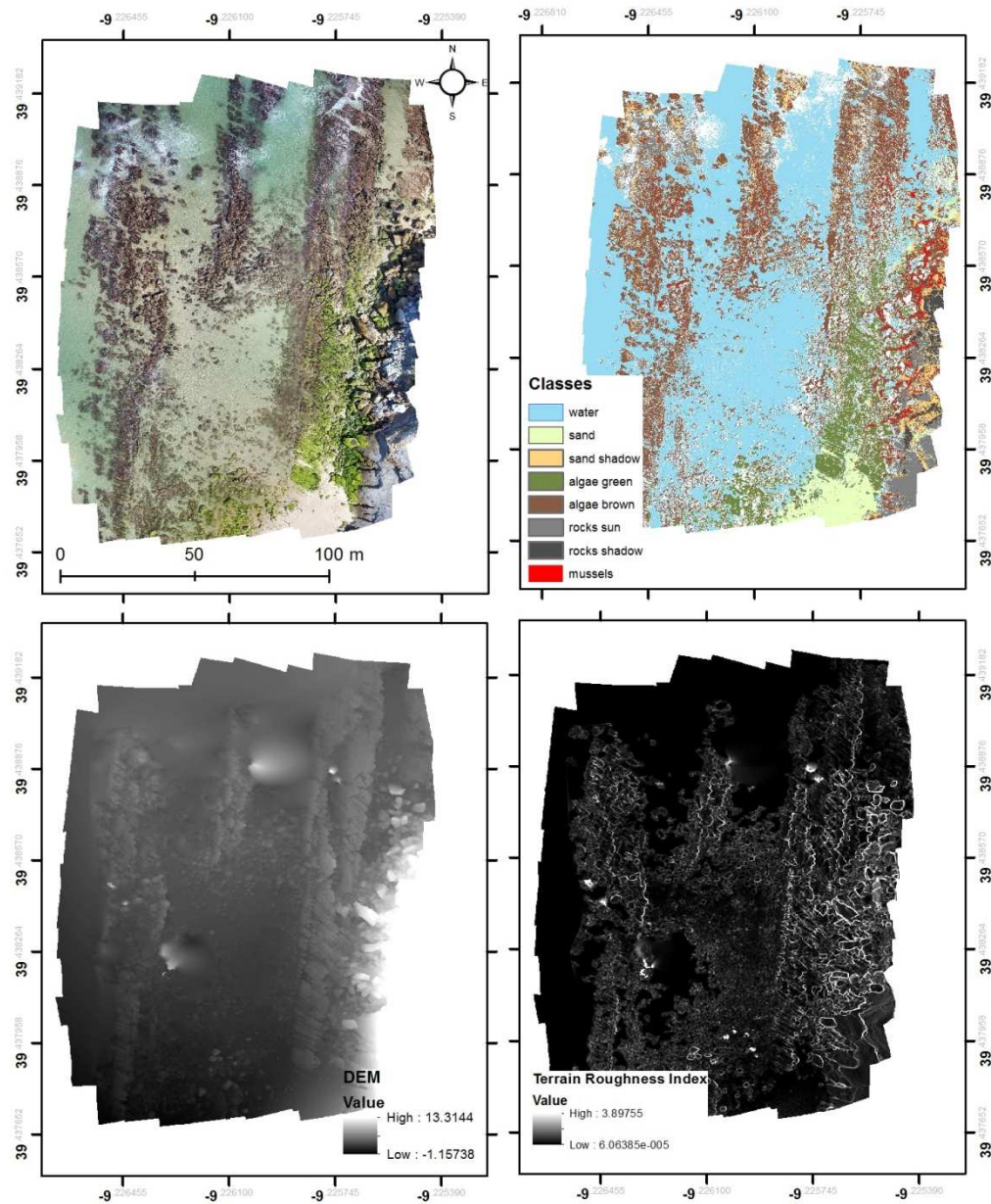


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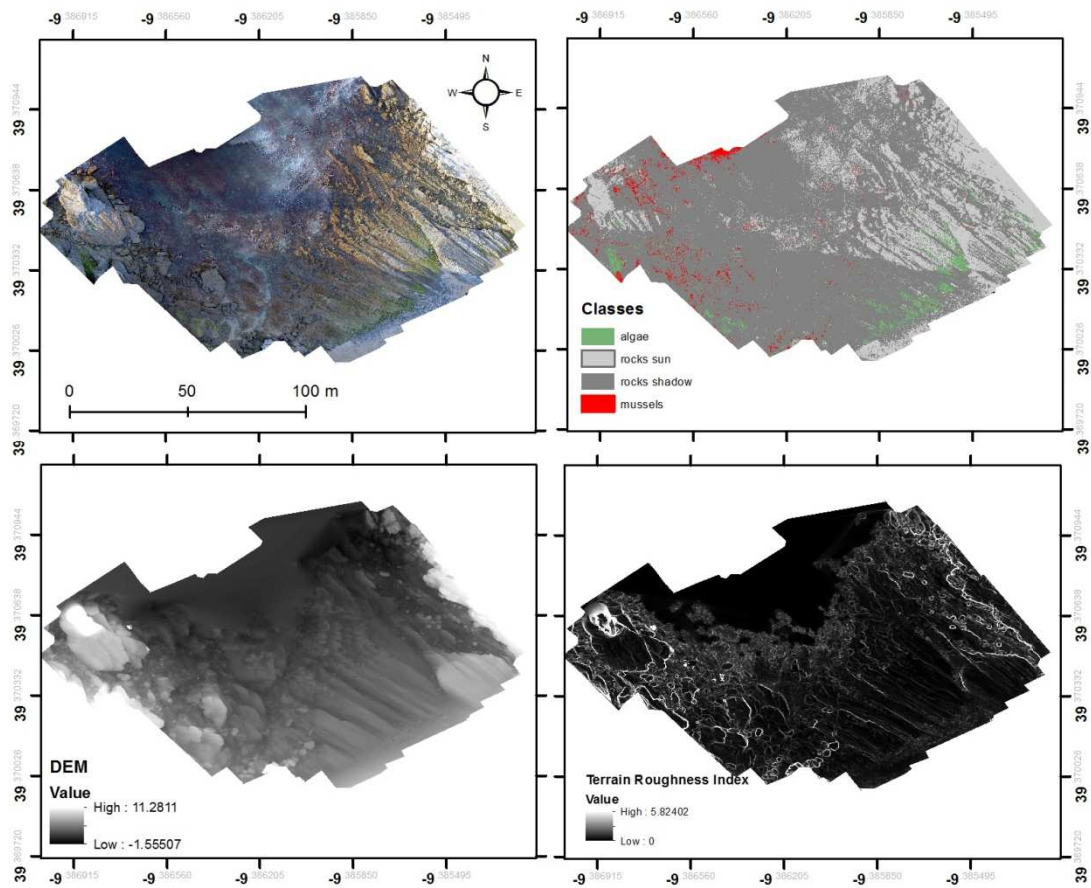
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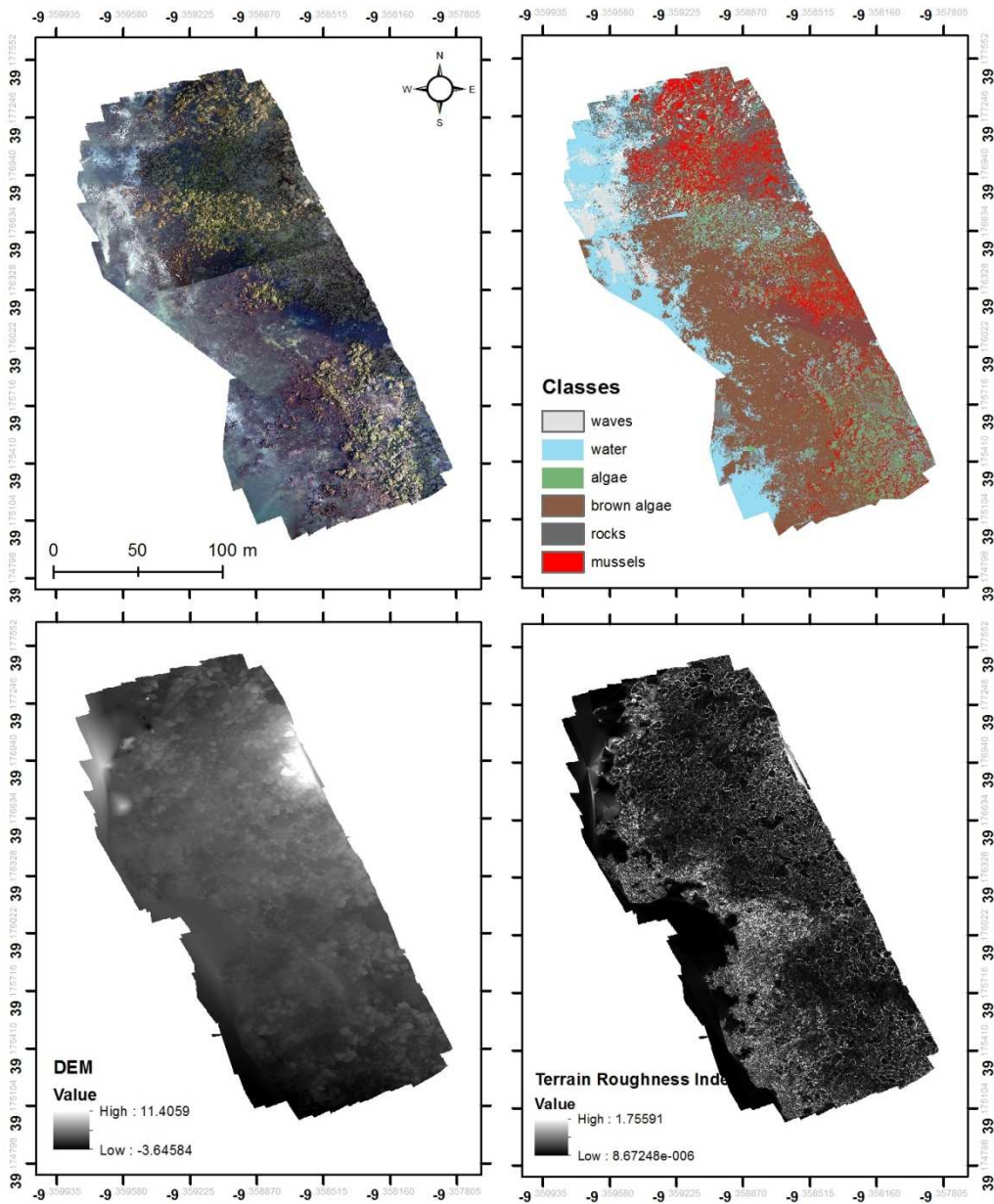
### 3.6 Supplementary Information 1 - Orthophoto mosaics, classified images, digital elevation models (DEM, in meters) and terrain roughness index, for all survey locations.



1 Foz Arelho

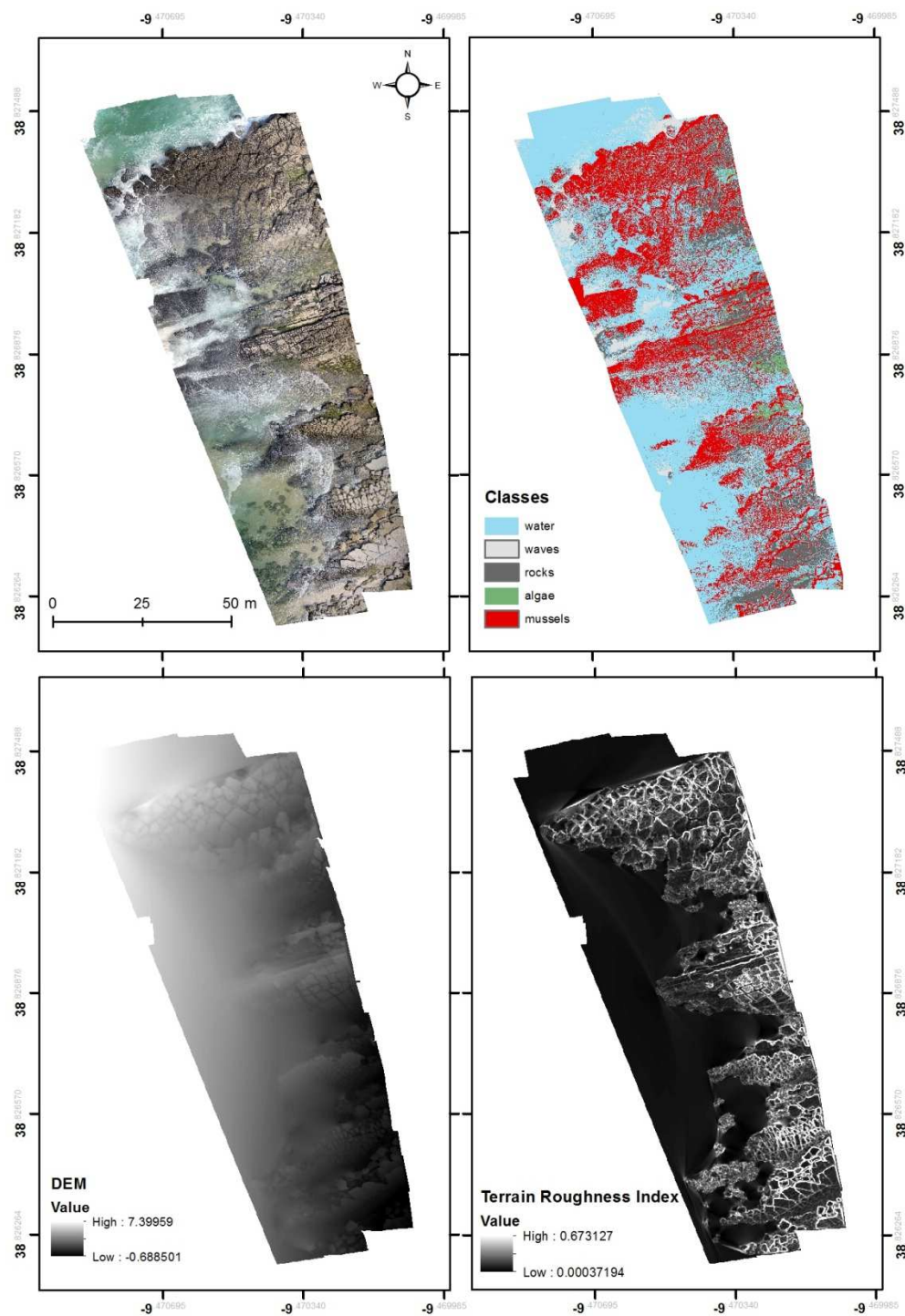


2 Peniche

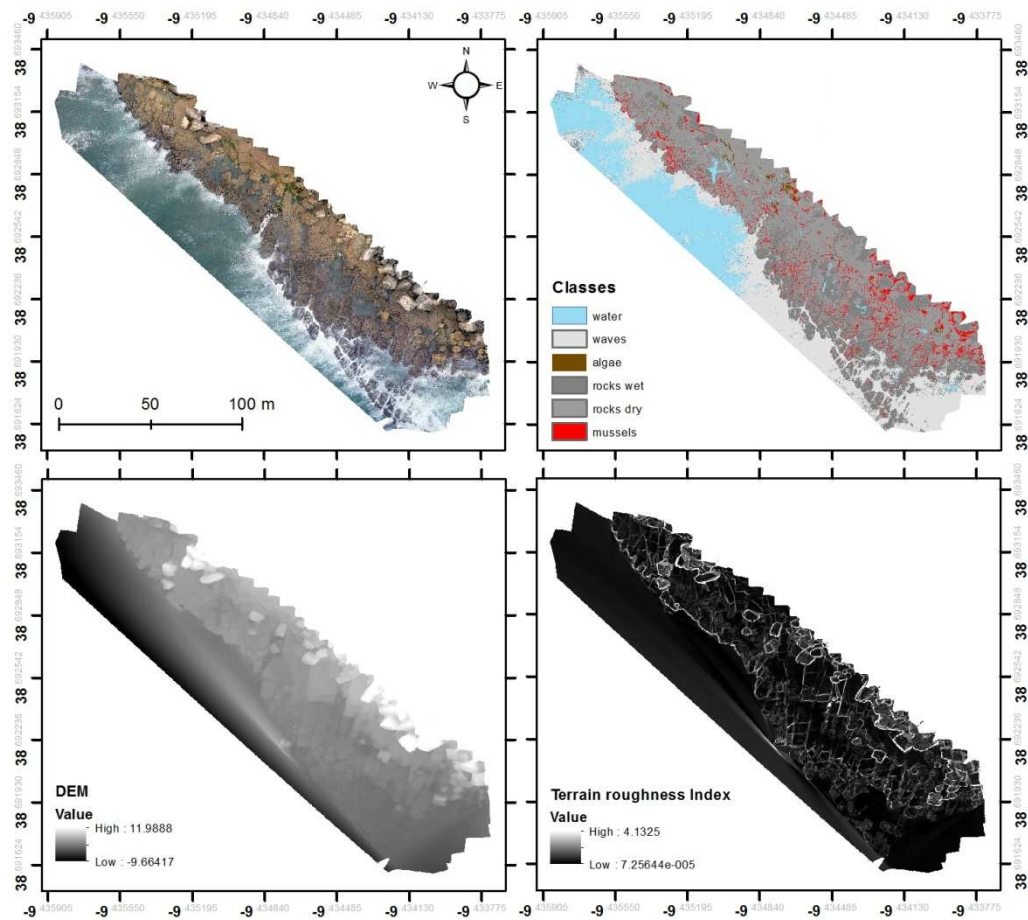


3 Porto Novo



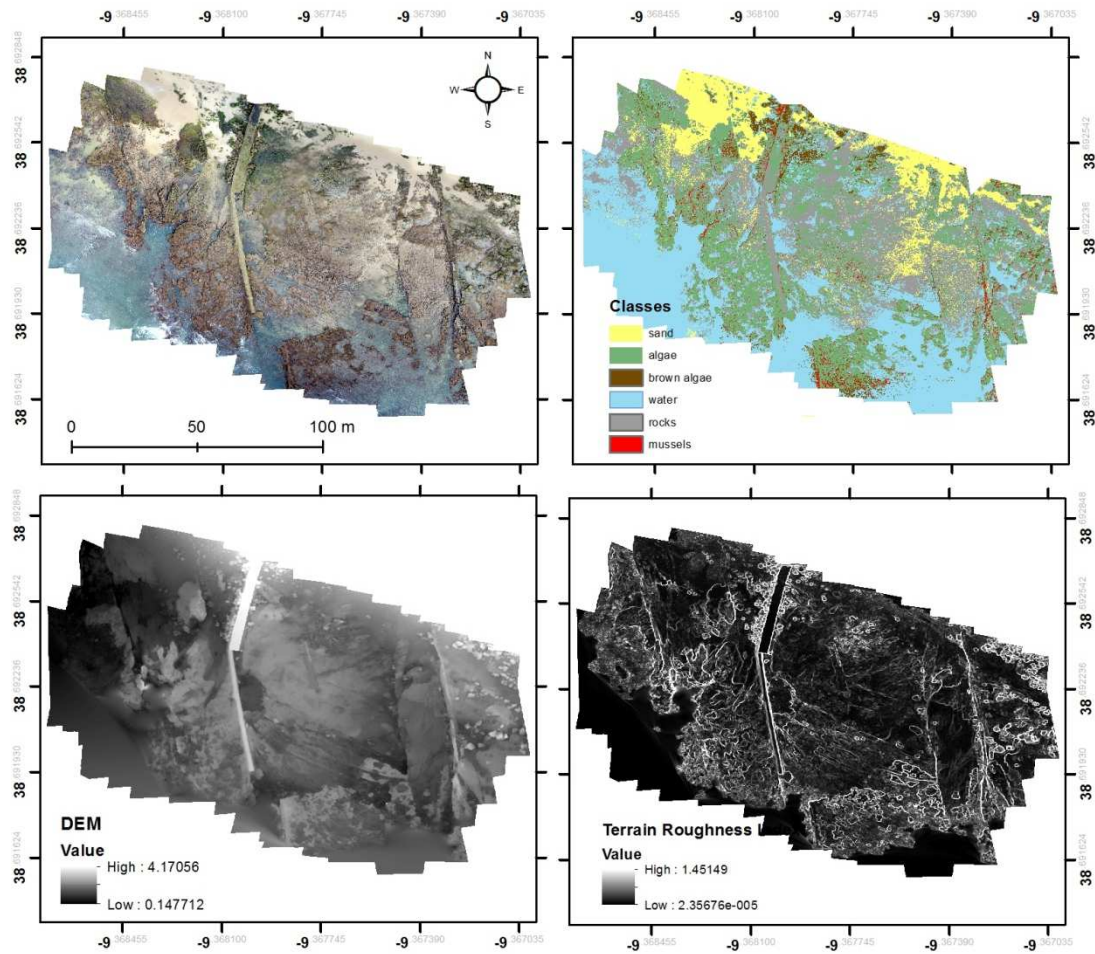


4 Maços

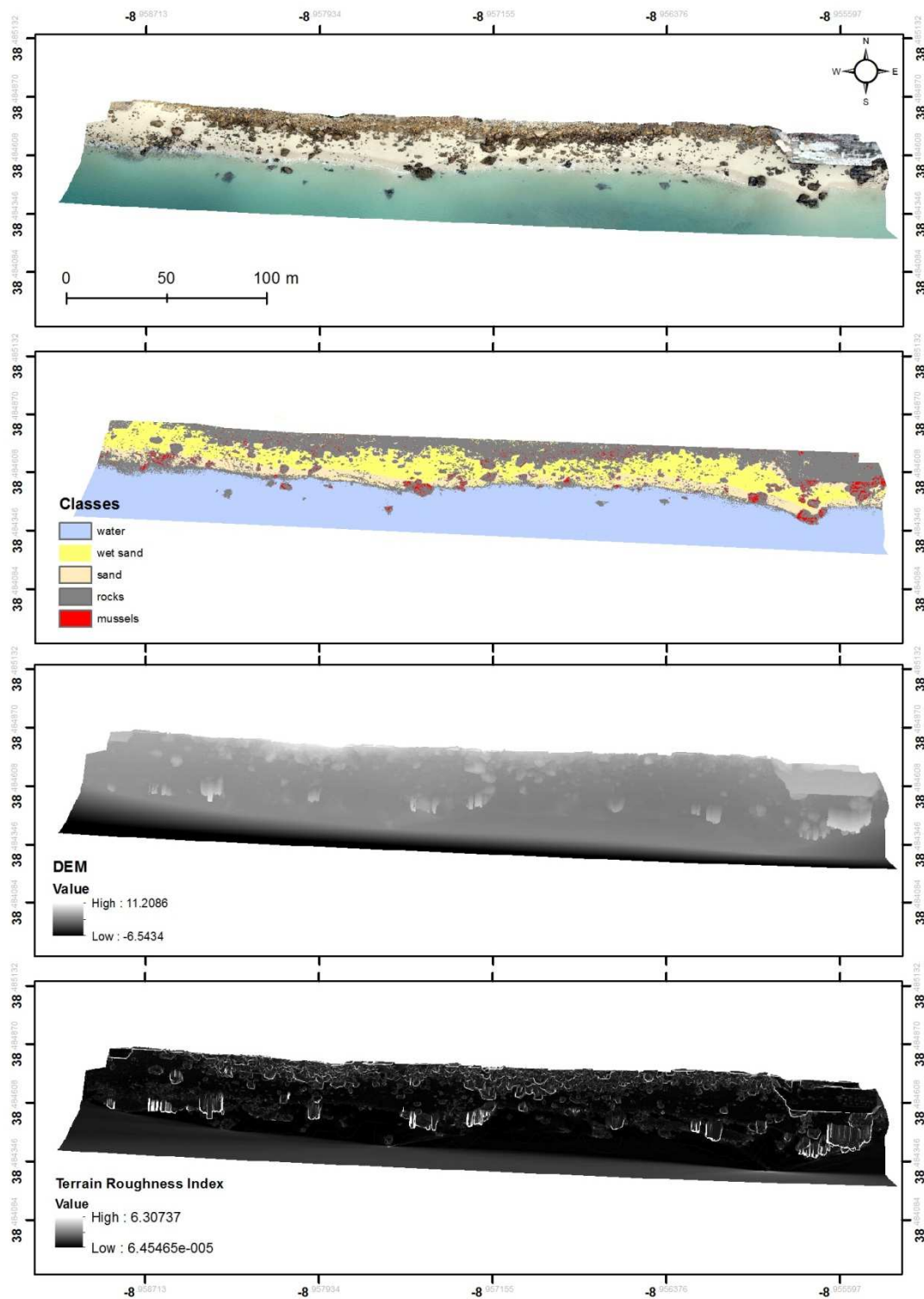


5 Mexilhoeira





6 Bafureira



7 Galapos

### 3.7 Supplementary Information 2 - Error matrices

Columns represent true classes, while rows represent the classifier's predictions. All correct classifications are along the upper-left to lower-right diagonal.

#### 1. Foz Arelho

		REFERENCE DATA							
PREDICTED	CLASS	WATER	SAND	SAND SHADOW	ALGAE GREEN	ALGAE BROWN	ROCKS SUN	ROCKS SHADOW	MUSSELS
	WATER	1,401	19	0	0	9	176	0	5
	SAND	0	1,452	0	0	1	179	0	2
	SAND SHADOW	0	0	998	0	69	22	777	87
	ALGAE GREEN	2	0	0	1,842	0	0	0	0
	ALGAE BROWN	0	5	7	4	1,656	103	160	309
	ROCKS SUN	0	26	6	0	53	1,202	32	182
	ROCKS SHADOW	0	0	28	0	24	32	1,403	9
	MUSSELS	0	0	0	0	38	0	114	1,562

#### 2. Peniche

		REFERENCE DATA				
PREDICTED	CLASS	ALGAE GREEN	ROCKS DRY	ROCKS WET	ALGAE BROW	MUSSELS
	ALGAE GREEN	1306	1	1	1	4
	ROCKS DRY	22	1782	80	8	2
	ROCKS WET	17	238	775	317	78
	ALGAE BROW	0	6	124	831	289
	MUSSELS	0	0	0	1	1199

#### 3. Porto Novo

		REFERENCE DATA					
PREDICTED	CLASS	WAVES	WATER	ALGAE	BROWN ALGAE	ROCKS	MUSSELS
	WAVES	744	2	6	0	42	0
	WATER	56	827	0	9	0	0
	ALGAE	0	0	1,022	211	0	2
	BROWN ALGAE	6	1	45	2,430	89	433
	ROCKS	0	0	0	362	1,573	0
	MUSSELS	0	0	8	277	298	1,197

## 4. Samarra

	CLASS	REFERENCE DATA				
		WATER	WAVES	SAND	ROCKS	MUSSELS
PREDICTED	WATER	1606	0	0	5	206
	WAVES	0	1124	0	0	0
	SAND	0	0	1638	43	1
	ROCKS	16	66	73	1912	183
	ALGAE	3	0	122	128	2269
	MUSSELS	0	0	15	133	398

## 5. Maçãs

	CLASS	REFERENCE DATA				
		WATER	WAVES	ROCKS	ALGAE	MUSSELS
PREDICTED	WATER	1,153.00		13	282	96
	WAVES	0	1,117.00		3	0
	ROCKS	0		73	1,607.00	8
	ALGAE	0	0	0	1,099.00	
	MUSSELS	1		0	211	498
						1,643.00

## 6. Mexilhoeira

	CLASS	REFERENCE DATA				
		WATER	WAVES	ALGAE	ROCKS DRY	ROCKS WET
PREDICTED	WATER	1158	0	0	0	0
	WAVES	57	1282	0	0	0
	ALGAE	0	0	806	0	0
	ROCKS DRY	180	9	1	1142	204
	ROCKS WET	188	0	49	193	1474
	MUSSELS	0	0	1	1	2

## 7. Bafureira

	CLASS	REFERENCE DATA					
		SAND	ALGAE	ALGAE BROWN	WATER	ROCKS	MUSSELS
PREDICTED	SAND	1144	7	0	2	19	1
	ALGAE	0	1991	23	3	111	138
	ALGAE BROWN	0	7	759	1	0	315
	WATER	5	5	21	1735	59	18
	ROCKS	48	109	4	117	1445	24
	MUSSELS	0	1	46	0	0	1940

## 8. Galapos

	CLASS	REFERENCE DATA				
		WATER	WAVES	ROCKS	ALGAE	MUSSELS
PREDICTED	WATER	1560	0	0	0	0
	WAVES	0	965	120	33	0
	ROCKS	0	35	1261	67	0
	ALGAE	0	2	202	3137	187
	MUSSELS	0	0	0	12	3586

Nolasco R, Gomes I, Peteiro L, Albuquerque R, Luna T, Dubert J, Swearer SE, Queiroga H. 2018 Independent estimates of marine population connectivity are more concordant when accounting for uncertainties in larval origins. *Scientific Reports*. 8(1) pp: 2641

**Abstract**

Marine larval dispersal is a complex biophysical process that depends on the effects of species biology and oceanography, leading to logistical difficulties in estimating connectivity among populations of marine animals with biphasic life cycles. To address this challenge, the application of multiple methodological approaches has been advocated, in order to increase confidence in estimates of population connectivity. However, studies seldom account for sources of uncertainty associated with each method, which undermines a direct comparative approach. In the present study we explicitly account for the statistical uncertainty in observed connectivity matrices derived from elemental chemistry of larval mussel shells, and compare these to predictions from a biophysical model of dispersal. To do this we manipulate the observed connectivity matrix by applying different confidence levels to the assignment of recruits to source populations, while concurrently modelling the intrinsic misclassification rate of larvae to known sources. We demonstrate that the correlation between the observed and modelled matrices increases as the number of observed recruits classified as unknowns approximates the observed larval misclassification rate. Using this approach, we show that unprecedented levels of concordance in connectivity estimates ( $r = 0.96$ ) can be achieved, and at spatial scales (20-40 km) that are ecologically relevant.

**Keywords:** larval dispersal, marine populations, numerical modelling, elemental fingerprinting, uncertainty

## 4.1 Introduction

The majority of marine macroinvertebrates and fishes have a biphasic life cycle comprised of relatively sedentary benthic adults and potentially dispersive pelagic larvae. Benthic populations of these species exhibit some degree of connectedness, with the consequence that local recruitment may be decoupled from local larval production. This creates challenges for identifying the drivers of population replenishment and persistence, which are fundamental to our understanding of gene flow, adaptation and evolution in the sea (Warner 1997), and for proper fisheries management and biodiversity conservation (Warner and Cowen 2002, Sale and Kritzer 2003). Additionally, variability in ocean circulation on the time frame of larval life (Siegel et al. 2008) and the lack of knowledge on biological parameters that interact with the circulation and other characteristics of the physical-chemical environment mean that predictions on the extent and direction of marine larval dispersal cannot be derived from first principles. Because of this limitation, available reviews and syntheses (Mora and Sale 2002, Thorrold et al. 2002, Levin 2006, Thorrold et al. 2007, Cowen and Sponaugle 2009, Burgess et al. 2014) advocate the use of multiple methods in order to increase confidence in empirical estimates of larval dispersal and population connectivity.

A variety of approaches have been applied to identify the origins and the destinations of pelagic marine larvae (Hellberg et al. 2002, Thorrold et al. 2002, Levin 2006, Werner et al. 2007, Thorrold et al. 2007, Hedgecock et al. 2007, Metaxas and Saunders 2009) and literature therein), which fall into four main groups: visual tracking of marine larvae, artificial tags, natural tags, and numerical biophysical modelling. Visual tracking of individual larvae is the only direct method available, but can only be applied to large larvae with short Pelagic Larval Durations (PLDs) and thus has limited applicability. The remaining techniques have been extensively used, although many lack general applicability because they are dependent on particular life-history traits, physiology or anatomy of the target taxon or species. All techniques have intrinsic uncertainties that depend on type of markers, analytical procedures and statistical methodology. A matter of concern is how these internal uncertainties affect the comparison among dispersal estimates when multiple methods are used.

A literature review based on 507 research articles published since 1990 (see additional information in Supplementary Information 1-Literature review for definitions, a classification of methodologies and references) indicates that 41 studies (Miller et al. 2005, Galindo et al. 2006, Baums et al. 2006, Dupont et al. 2007, Bradbury et al. 2008, Piggott et al. 2008,



Chiswell 2009, Jolly et al. 2009, Salas et al. 2009, Liu et al. 2010, White et al. 2010, Kool et al. 2010, Berumen et al. 2010, Galindo et al. 2010, Selkoe et al. 2010, Kool et al. 2011, Alberto et al. 2011, Schunter et al. 2011, Foster et al. 2012, Berry et al. 2012a, Berry et al. 2012b, Correia et al. 2012, Domingues et al. 2012, Soria et al. 2012, Ben-Tzvi et al. 2012, Coscia et al. 2012, Crandall et al. 2012, Di Franco et al. 2012, Thomas and Bell 2013, Jolly et al. 2013, Moksnes et al. 2014, Cook et al. 2014, Simpson et al. 2014, Sunday et al. 2014, Schiavina et al. 2014, Davies et al. 2014, Nanninga et al. 2015, Fraker et al. 2015, Young et al. 2015, Thomas et al. 2015, Gormley et al. 2015) have used at least two methodologies to estimate marine larval dispersal and connectivity matrices. The two most common approaches have been to use genetic markers and a numerical biophysical model, or the micro-chemistry of hard parts and a numerical biophysical model, but genetic markers and micro-chemistry, and combinations of genetic markers or micro-chemistry with current measurements, have also been employed. The review indicates that the degree of convergence between the different methods is widely taken as a measure of the trust that is put on the final solution: the more convergent the different methods, the higher the confidence on the description of the dispersal process. The majority of these assessments were qualitative, expressed as verbal descriptions of the patterns of dispersal that were obtained, with particular emphasis on the spatial coincidence of observed or predicted barriers to dispersal. A variety of methods were employed to produce semi-quantitative assessments (different approaches tested separately for significance, followed by numerical comparison of the test statistics) and quantitative assessments (a test statistics of the fit between the dispersal estimated by the different approaches was calculated and assessed), depending on the type of dispersal metrics that was employed: assessments of proportional variability explained by separate observed and predicted genetic isolation-by-distance (Alberto et al. 2011) or by separate isolation-by-geographic distance and isolation-by-oceanographic distance regressions (White et al. 2010, Davies et al. 2014, Thomas et al. 2015), Mantel tests between observed and/or predicted distance matrices (Dupont et al. 2007, Foster et al. 2012, Berry et al. 2012a, Sunday et al. 2014, Young et al. 2015), log Bayes factors analysis that the predicted genetic structure fits the observed genetic structure (Crandall et al. 2012), sums of squared differences between predicted and observed allele frequencies (Galindo et al. 2010), multiple regression of genetic distance on oceanographic distance and environmental variables (Selkoe et al. 2010), MANOVA of elemental ratios of individuals assigned to groups based on parentage (Berumen et al. 2010), and correlation between connectivity matrices (Schunter et al. 2011).

An important consideration on the use of empirical methods or models to infer dispersal and population connectivity is the confidence on the assignment to the population of origin. The empirical methods used by previous studies assign larvae or recruits to putative parental populations on a probabilistic fashion (based on assumptions of probability distributions of alleles or elements, number and size of populations, and other demographic processes), and have intrinsic uncertainties (Kaplan et al. 2016). Three studies that did estimate a connectivity matrix based on genetics or elemental fingerprinting did explicitly incorporate this uncertainty into the decision of allocating larvae or recruits to parental populations, by specifying a posterior probability threshold for correct assignment (from 0.70 to 0.95; (Schunter et al. 2011, Simpson et al. 2014, Fraker et al. 2015), while five studies simply allocated larvae or recruits to a given population when the posterior probability of pertaining to this population was higher than that of pertaining to any other population (Miller et al. 2005, Bradbury et al. 2008, Alberto et al. 2011, Thomas and Bell 2013, Nanninga et al. 2015). Numerical biophysical models also have intrinsic uncertainties associated with different biological and oceanographic causes (Werner et al. 2007, Metaxas and Saunders 2009). Typically, the studies reviewed here provided some kind of temporal integration or used multiple runs with different environmental forcings, in order to smooth seasonal and inter-annual variability in currents. None of the studies provided information on sensitivity of the model to parameterization of sub-grid processes, nesting or resolution, although several of the studies were based on oceanographic models that have been extensively tested elsewhere (e. g. Galindo et al. 2006, Kool et al. 2010, 2011, Berry et al. 2012a, 2012b, Domingues et al. 2012). Most studies assumed fixed values for biological parameters, based on literature data, although a few used different biological scenarios in separate runs of the model.

Advancements on the merging of independent approaches to describe dispersal patterns have been to use connectivity matrices derived from biophysical models into population genetic models, in order to predict genetic structure. If the predicted genetic structure matches the observed structure, a case is made that migration mediated by oceanographic patterns of propagule transport influences gene flow. These studies used a derivation of the Bodmer and Cavalli-Sforza (Bodmer and Cavalli-Sforza 1968) matrix model of migration to predict equilibrium allele frequencies after a variable number of generations (White et al. 2010, Kool et al. 2010, 2011, Foster et al. 2012, Young et al. 2015), or used modelled pairwise migration probabilities to inform a population model predicting allele frequencies at equilibrium (Galindo et al. 2006, 2010, Sunday et al. 2014).

Most studies reviewed above used numerical biophysical models to obtain independent estimates of dispersal that could either be compared to empirical estimates, or that could feed population genetic models. None of the studies presented the models in a framework of model validation against observations, nor were they concerned with the uncertainty inherent to the empirical measurements of connectivity when comparing predictions of the models to empirical observations (Hannah 2007, Bellocchi et al. 2010). Only three studies explicitly accounted for uncertainty into the decision of allocating larvae or recruits to parental populations (Schunter et al. 2011, Simpson et al. 2014, Fraker et al. 2015), and only (Schunter et al. 2011) attempted a formal quantitative comparison between model predictions and observations. This uncertainty can be very large and probably depends on the number of populations. In Schunter et al. (2011), which included 13 populations, 68% (262 in 382) individuals were discarded by applying a threshold level for correct assignment of 80%. In Simpson et al. (2014), which considered only two populations, slightly less than 20% of the individuals were classified as unknowns, for a 0.95 probability of correct allocation.

Our review of the literature indicates that many of the studies did not use stringent rules to assign dispersers to their natal populations based on their probabilities of correct assignment, and when they did they did not investigate why these probabilities might vary, nor how the confidence level used would affect comparison among estimates. Thus, there is a clear need to explicitly address the challenges of comparing dispersal estimates across methods while addressing the issue of uncertainty in order to i) reduce this uncertainty wherever possible and ii) demonstrate that the convergent solution provides a robust estimate of the connectivity matrix.

In the present paper we addressed this issue in the Mediterranean mussel, *Mytilus galloprovincialis* Lamarck, using elemental fingerprinting and a numerical biophysical model. Our geographical domain is the west coast of the Iberian Peninsula. To do so we manipulated the observed (empirically-derived) connectivity matrix by applying different confidence levels to the assignment of recruits to the source populations. Recruits that failed to pass the prescribed confidence level were assigned to an unknown category. We manipulated the modelled connectivity matrix by using different population and larval biology scenarios. Moreover, we simulated the intrinsic variability of the geochemical signal by classifying modelled recruits as unknowns in a proportion equivalent to the misclassification rate of the larvae to their own sources, which is a measure of the inherent variability of the elemental profile. A second source of uncertainty was addressed by also

classifying as unknowns the modelled recruits that originated outside the region for which elemental data was available. We demonstrate that the degree of convergence between the observed and modelled matrices increased as the proportion of recruits classified as unknowns approached the modelled proportion of unknowns, and that the increase in convergence is significantly different from that obtained with a random classification of recruits into an unknown origin.

## **4.2 Methods**

### **4.2.1 Elemental fingerprinting and the generation of observed connectivity matrices**

The methodology used to obtain an atlas of geochemical natal signatures and for establishing the natal origin of the recruits is described in Gomes et al. (2016). In brief, this methodology consisted of growing early laboratory-produced mussel embryos for 6 days inside incubators deployed in the field until a larval shell had clearly developed (70 to 140  $\mu\text{m}$  shell length). Incubators were deployed at approximately 20 km intervals along the central coast of Portugal (Fig. 4.1), which is characterized by extensive rocky shores and is delimited by long stretches of almost continuous sandy shores to the north (150 km) and south (50 km). Six weeks after the start of the incubations, mussel juveniles were collected from rocky shores adjacent to each incubation site. Given the expected larval and juvenile growth rates at the temperature recorded during the study period (June-July of 2013), the time window of larval incubation should coincide with the period when the sampled recruits were produced. Larval shells and the larval portion of the recruits' shells were then subjected to LA-ICPMS analysis using standard protocols (see Gomes et al. (2016) for detailed methodology).

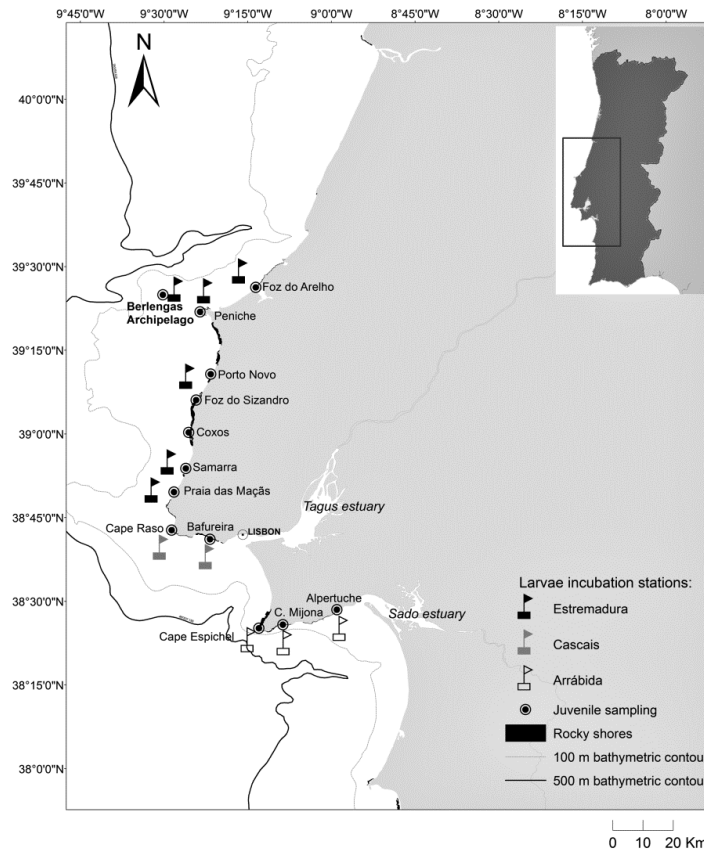


Figure 4.1 - Map of larval incubation stations and juvenile sampling sites. Estremadura North: Berlengas, Peniche and Foz do Arelho. Estremadura South: Porto Novo, Samarra and Praia das Maças. Cascais Bay: Cabo Raso and Bafureira. Arrábida Bay: Cabo Espichel, Cova da Mijona and Alpertuche. For better visualization purposes, moorings in the map are illustrated more offshore than in the field (deployed at a depth of 15 to 20 m). Adapted from Gomes et al, 2016.

A jack-knifed linear Discriminant Function Analysis (DFA) of element-to-calcium ratios applied to the larval data produced a relatively low reclassification success at the site level (43.7% of cross-validated cases correctly classified), but a better discrimination at the region level (79.5%) when considering three regions: Estremadura (sites Berlengas, Peniche and Foz do Arelho, Porto Novo, Samarra and Praia das Maças), Cascais Bay (sites Cabo Raso, Bafureira) and Arrábida Bay (sites Cabo Espichel, Cova da Mijona, Alpertuche). An intermediate reclassification success (68.3%) was obtained when considering four regions, by splitting the large Estremadura region into two: Estremadura North (Berlengas, Peniche and Foz do Arelho) and Estremadura South (Porto Novo, Samarra and praia das Maças). A Monte-Carlo cross-validation technique (Simmonds et al. 2014) indicated that randomly discarding up to 80% of the larvae did not have significant effects on the misclassification error relative to the full data set, confirming the capability to detect distinctive signatures

for each region and sufficient sampling effort to account for variability within each region (Simmonds et al. 2014).

The discriminant functions trained with the larval data were then used to assign recruits to natal origins, at the regional level, and to generate a series of observed connectivity matrices that differed depending on the confidence level applied during the assignment procedure. DFA assigns objects to previously defined groups based on the multivariate probability distribution of the dependent variables across objects within each group (Quinn and Keough 2002). DFA calculates the posterior probabilities of each object belonging to each group and assigns an object to a specific group if the probability of pertaining to that group is higher than the probability of pertaining to the remaining groups, independently of the magnitude of probability differences. In the present case this introduces a source of uncertainty associated with the inter-individual variability of the elemental profile, which may result in incorrectly assigned recruits (Type 2 recruits; see below). Additionally, when assigning objects to groups DFA assumes that all objects belong to one of the *a priori* defined groups, and to none other. Our data set presumably violates this assumption because there is the possibility that recruits could have originated from outside the sampled region (Type 3 recruits; see below), although this should be minimized by the isolation of the sampled region by long stretches of coastline devoid of mussels. In order to account for these inherent types of uncertainty we used different confidence levels during the assignment procedure (Assignment Probability Thresholds, APT), based on the posterior probability thresholds of originating from the different populations: better-than-the-rest (none of the recruits classified as of unknown origin; recruits assigned to the population to which they have the better probability of belonging), 0.50, 0.75, 0.90, 0.95 and 0.99 (Table 4.1). These APT cover the range of confidence levels used in most practical applications and allowed us to test the sensitivity of the compliance between observed and modelled connectivity matrices to the confidence level used for generating the observed connectivity matrix.

Table 4.1 Definitions and codes of types of recruits, spawning regimes, larval behaviours, matrix spatial arrangements and assignment probability thresholds.

<b>Types of recruits or Scenarios</b>	<b>Code</b>
<b>Types of recruits</b>	
Recruits originating within the core region that are positively assigned to a specific origin.	Type 1
Recruits originated within the core region but of uncertain origin because of a natal signature not distinct enough to warrant a positive assignment to a specific origin.	Type 2
Recruits originated outside the core region and of unknown origin because of an unknown natal signature.	Type 3
<b>Spawning regimes</b>	
Continuous larval emission during each high tide until July 12.	S1
Continuous larval emission during each high tide until June 30; from that day on, discontinuous larval emission, skipping one of every two high tides until July 12.	S2
Continuous larval emission during each high tide until June 30; from that day on, discontinuous larval emission, skipping two of every three high tides, until July 12.	S3
Continuous larval emission during each high tide until July 1; from that day on, no more larvae were released.	S4
<b>Larval behaviours</b>	
Passive larvae.	Pa
Ontogenetic migration from a depth around 5 m until the pediveliger stage, followed by a migration to a depth around 12.5 m.	Om
Larvae dwelling in the bottom layer in shallow water and from 30 - 50 m deeper water.	Bl
<b>Spatial arrangements</b>	
Origins: Estremadura, Cascais Bay and Arrábida Bay. Destinations: Estremadura, Cascais Bay and Arrábida Bay.	3x3
Origins: Estremadura, Cascais Bay and Arrábida Bay. Destinations: Estremadura North, Estremadura South, Cascais Bay and Arrábida Bay.	3x4
Origins: Estremadura North, Estremadura South, Cascais Bay and Arrábida Bay. Destinations: Estremadura North, Estremadura South, Cascais Bay and Arrábida Bay.	4x4
<b>Assignment Probability Thresholds</b>	
None of the recruits classified as of unknown origin; recruits assigned to the population to which they have the better probability of belonging.	Better-than-the-rest
Recruits classified as of unknown origin if the highest posterior probability of assignment was lower than the indicated value; otherwise, assigned to the population to which they have the better probability of belonging.	0.50, 0.75, 0.90, 0.99

#### 4.2.2 Biophysical numerical model and the generation of modelled connectivity matrices

The biophysical numerical model included two components: a nested oceanographic model based on the Regional Ocean Modelling System (ROMS), which produced velocity and temperature fields at 1 h intervals; and a biological model, implemented through a Lagrangian offline model that simulated the spatial and temporal distribution of mussel spawning, larval vertical migration behaviour, temperature-dependent planktonic larval duration and larval trajectories, based on the stored ROMS velocity and temperature fields interpolated at 300 s intervals. The nested model included a large domain extending from 12.5°W to 5.5°W and 34.4°N to 45.5°N (resolution of 1/27°; 60 vertical levels), which was used to provide boundary conditions to a medium domain corresponding to the West Iberian Margin (WIM; Cape St Vincent at 37° N to Cape Finisterre at 43° N, and from 11.5° W to the WIM coast at 8.5° W; resolution 1/60°; 45 levels). The medium domain was the target domain used for the dispersal simulations and was connected by two-way nesting to a small domain (from Figueira da Foz at 40.2° N to Sines at 37.8° N, extending to 10.5° W; resolution 1/180°, 45 levels), which encompassed the main region where natal and recruit signatures were collected (Fig. 4.1). A number of larvae proportional to the mussel biomass at each segment of the coast (Siregar 2014) and to seasonal spawning activity (Philippart et al. 2012) was released adjacent to each rocky shore cell of the model and allowed to grow at a rate dependent on the thermal history predicted by ROMS, until a competent phase was reached (Ruiz et al. 2008, Pettersen et al. 2010). If a larva found a rocky shore cell during the competent phase it was allowed to recruit; otherwise it would die. Because numerical models poorly resolve the coastal boundary layer where non-linear processes predominate (Nickols et al. 2012), a coastal buffer strip of 3 cells along the rocky shore was used as a settlement habitat. A more complete account of the biophysical model, environmental forcing and validation information based on (Bayne 1964, Chia et al. 1984, Lutz and Kennish 1992, Villalba 1995, Caceres-Martinez and Figueras 1998, Mcquaid and Phillips 2000, Metaxas 2001, Egbert and Erofeeva 2002, Qiu et al. 2002, Phillips 2002, Pernet et al. 2003, Shanks and Brink 2005, Shchepetkin and McWilliams 2005, Peliz et al. 2007, Ruiz et al. 2008, Skamarock et al. 2008, Carr et al. 2008, Rilov et al. 2008, Sameoto and Metaxas 2008, Oliveira et al. 2009, Pettersen et al. 2010, Daigle and Metaxas 2011, Fuchs and DiBacco 2011, Domingues et al. 2012, Sanchez-Lazo and Martinez-Pita 2012, Philippart et al. 2012, Nolasco et al. 2013, Nolasco, Pires, et al. 2013, Gomes et al. 2016) can be found in the Supplementary Information 2-Biophysical model.



#### **4.2.3 Accounting for uncertainty: recruit origin and the construction of observed and modelled connectivity matrices**

The Observed connectivity matrix refers to the geographical area for which natal and recruit elemental fingerprints were collected. The biophysical model covers a wider region, with additional origin and destination populations. Therefore, the Modelled connectivity matrix is larger than the Observed connectivity matrix. In the following description, whenever we refer to the core connectivity matrix(es) we are referring to the area from where elemental fingerprints were sampled.

When constructing the Observed connectivity matrix, the decision on the assignment of each recruit to a particular population of origin depends on the confidence level we wish to put in the assignment, i. e. depends on the selected posterior probability threshold of pertaining to that specific origin. With a higher confidence level we increase the number of unassigned recruits. In each particular case the unassigned individual has one of two possible origins: it may have originated within the core region but the elemental fingerprint of the origin is not distinct enough to warrant a positive assignment to the source population (Type 2 recruits in Fig. 4.2); or it may have originated from a population outside the core region (Type 3 recruits in Fig. 4.2). Type 2 recruits should be part of the connectivity core matrix but have to be assigned to an unknown origin. Type 3 recruits are not part of the core connectivity matrix because they originated outside the core region. They are also assigned to an unknown origin, because the natal signature of the population of origin is unknown. Type 1 recruits are those that are positively assigned to a specific origin population in the core matrix (see Fig. 4.2).

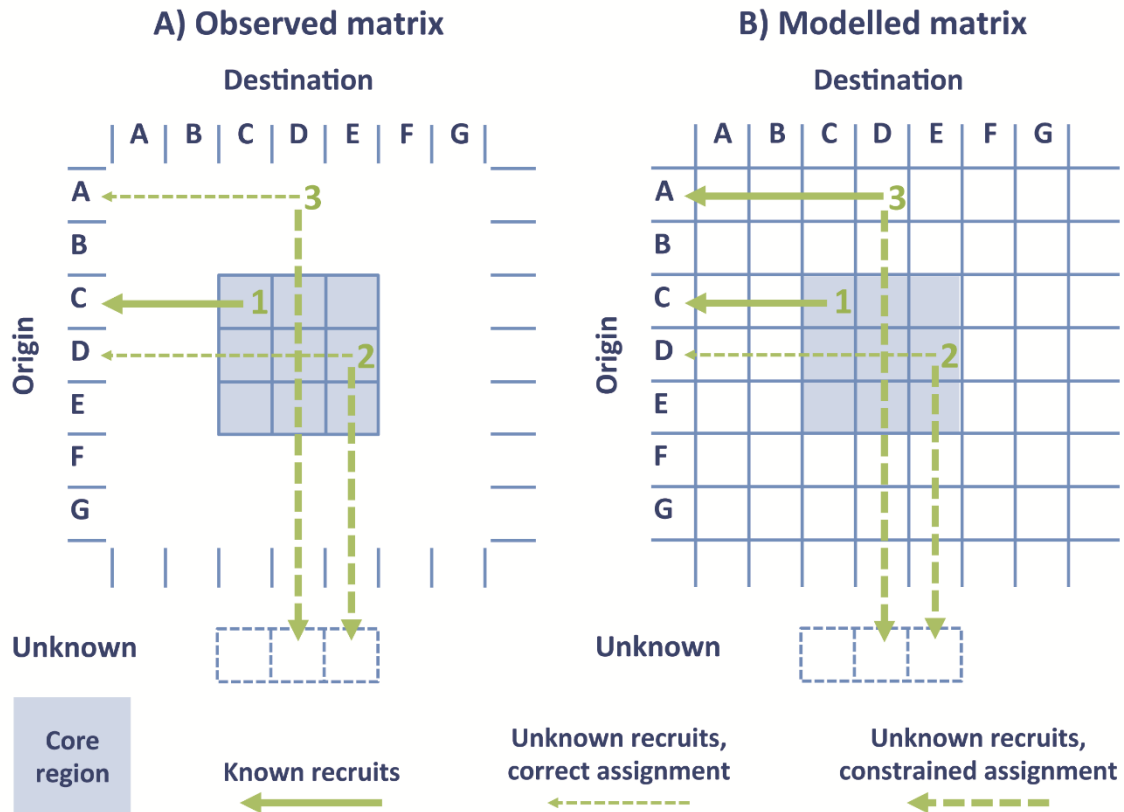


Figure 4.2 Observed (A) and Modelled (B) connectivity matrices for the 3 by 3 subdivision of the core region. The arrows illustrate the assignment of recruits into the populations of origin. Type 1 recruits (1): individuals recruited into the core region that originate within the core region and are assigned to origins within the core region. Type 2 recruits (2): individuals recruited into the core region that originate within the core region; assignment in the Observed matrix is not possible because of a poorly defined natal fingerprint and they are classified as unknowns; in the Modelled matrix they are classified as unknowns based on the probability of incorrect self-assignment of the larvae. Type 3 recruits (3): individuals recruited into the core regions that originate outside the core region; assignment in the Observed matrix is not possible because of an unregistered natal fingerprint and they are also classified as unknowns; in the Modelled matrix they are classified as unknowns to simulate the lack of knowledge about their natal signature.

The Modelled, connectivity matrix is not affected by these sources of uncertainty because all recruits, irrespective of their origin and destination, can be "tracked back" by the model to their original populations (actually they are tracked forward from origin to destination or death). We simulated the uncertainty in the observations caused by the fact that the natal elemental signature is not distinctive enough to allow a positive assignment in all cases. To do this we assigned an unknown origin to a number of recruits into the core region that originated inside the core region, proportionally to the misclassification rate of the larvae. This forced some of the modelled recruits into Type 2 (see Fig. 4.2). All modelled recruits

originating outside the core region but recruiting here are Type 3 recruits and not part of the connectivity matrix by definition (see Fig. 4.2). We also assigned these individuals to an unknown origin in order to simulate the lack of knowledge about their natal signature. Based on the observed elemental fingerprints a few of them would falsely be assigned to an origin within the core region because of an unclear natal fingerprint. This uncertainty cannot be simulated. We could predict the proportion of the modelled recruits that should falsely be classified into the core region based on the misclassification rate of the larvae (by assuming an average value of this rate for the whole area), but there is no way of predicting to which population of the core region these recruits should be assigned to. We assume this source of uncertainty is negligible because: i) the further away from the core area the likelier that the natal signatures differ from those of the core area, reducing the probability of falsely assigning these recruits to an origin inside the core area; and ii) there are long stretches of sandy shores to the north and south of the core area, effectively reducing the number of Type 3 recruits.

Given the above, we generated a series of Observed connectivity matrices that differed (see below) in the number of the partitions of the core region (3 different arrangements) and confidence level (6 levels). We also generated a series of Modelled connectivity matrices that differed (see below) in spawning regime (4 regimes), larval behaviour (3 behaviours) and partitioning of the core region (3 different arrangements). We corrected the core Modelled matrix for Type 2 recruits by subtracting from the predicted recruits in each cell a number proportional to the misclassification rate of the corresponding origin. Each row of the Modelled core matrix was therefore corrected by a different proportion. Modelled Type 2 and Type 3 recruits were included in an unknown row. Observed recruits that failed to pass the confidence level threshold were also included in an unknown row. In the above comparisons, Observed and Modelled matrices were standardized by dividing the number of recruits into each destination by the total number of recruits that settled into that destination, i. e. by the sum of the respective column. The rationale for this standardization is that the sampling of recruited individuals was constrained to an approximately constant number of individuals in each location, and did not reflect the distribution of settlement intensity among the sites. In contrast, the number of recruits predicted by the biophysical model did reflect the distribution of settlement intensity, because it incorporates not only the pattern of connectivity, but also the total number of larvae "hatched" in the model. That standardization allowed us to compare relative numbers of recruits into each destination originating from the different origins in both matrices.

#### 4.2.4 Accounting for uncertainty: mussel biology scenarios

In order to bracket the uncertainty regarding larval production and behaviour, we considered 4 scenarios of spawning regime and 3 scenarios of larval behaviour, and ran the biophysical model for all 12 combinations. The spawning regime scenarios attempted to simulate the reproductive exhaustion of individuals subsequent to the peak of gamete emission in spring/early summer described for the Iberian Peninsula, as described by Suárez et al. (2005) and Philippart et al. (2012). Thus, the different regimes (Table 4.1) included constant larval spawning during high tide (mussels spawn only when submersed) along the entire rocky shore coast proportionally to population density during spring and early summer, followed by a progressive decline in larval emission towards the end of July, according to the following criteria: (S1) continuous larval emission during each high tide until July 12; (S2) continuous larval emission during each high tide until June 30; from that day on, discontinuous larval emission, skipping one of every two high tides until July 12; (S3) continuous larval emission during each high tide until June 30; from that day on, discontinuous larval emission, skipping two of every three high tides, until July 12; and (S4) Continuous larval emission during each high tide until July 1; from that day on, no more larvae were released. The larval behaviour scenarios (Table 4.1) included: (Pa) completely passive larvae, as implied by Mcquaid and Phillips (2000); (Om) an ontogenetic migration from a depth around 5 m until the pediveliger stage, followed by a migration to a depth around 12.5 m, according to studies suggesting larvae tend to migrate deeper in the water column during development (Rilov et al. 2008, Fuchs and DiBacco 2011); and (BI) larvae dwelling in the bottom layer in shallow water and from 30 to 50 m in deeper water; this unrealistic scenario was intended to provide a contrast to the other two scenarios.

#### 4.2.5 Arrangement of the core matrix

We used 3 arrangements of the core connectivity matrix (Table 4.1) that were derived from *a priori* considerations about the oceanography and geometry of the region (which includes open coasts, capes, bays and coastal mountains), which can influence the probability of imprinting distinctive natal signatures (Levin 2006, Thorrold et al. 2007). The first was a 3x3 arrangement, with sampling sites for both origin and destination grouped into Estremadura, Cascais Bay and Arrábida Bay. This arrangement is based on the expectation of a distinct signature in the bays, caused by the influence of the Tagus and Sado rivers, and of a homogeneous signature along the more exposed Estremadura coast. In the second (3x4) and third (4x4) scenarios we kept the Cascais and the Arrábida bay regions, but made a

distinction between the Estremadura North and South sections, separated by Cape Carvoeiro. This major cape induces strong and recurrent filament activities in response to upwelling events, which affect local oceanography and decouple to some degree both sections of the coast (Oliveira et al. 2009, Cordeiro et al. 2015). In the second scenario we expect a common natal signature for the whole Estremadura coast, but distinct settlement zones (Estremadura North and South) due to a two-cell circulation caused by the topographic influence of the cape. The third scenario considers the Estremadura North and South partition for both emission and settlement zones, based on the expectation of distinct natal signatures and circulation cells.

### **4.3 Results**

#### **4.3.1 Generation of observed and modelled connectivity matrices**

The distributions of posterior probabilities of mussel recruits pertaining to each of the putative origins differed markedly among regions, for both the 3-region (Fig. 4.3) and 4-region (Fig. 4.4) connectivity matrices. In both cases Arrábida Bay was the most important source, with either 62 (APT - 0.99) or 82 (APT - 0.90) recruits originating from this region, when considering 3 regions, and either 61 (APT - 0.99) or 83 (APT - 0.90) recruits originating from this region, when considering 4 regions. In contrast, the number of recruits with assignment probabilities  $<0.90$  was very similar among regions in the case of 3 regions (26, 26 and 25 for Arrábida, Cascais and Estremadura), but considerably more variable in the case of 4 regions (28, 20, 5 and 46 for Arrábida, Cascais, Estremadura North and Estremadura South). Thus, largely regardless of the method applied, Arrábida Bay was the main source of recruits to the different regions during the period of the study (see also Simmonds et al. 2014).

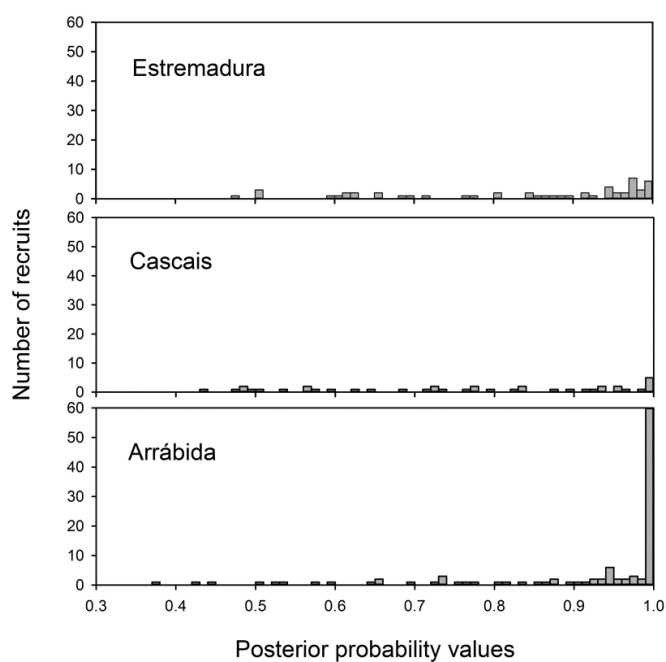


Figure 4.3 Posterior probabilities of assignment of mussel recruits into three putative origins, based on linear discriminant functions trained with larval shell elemental profiles.

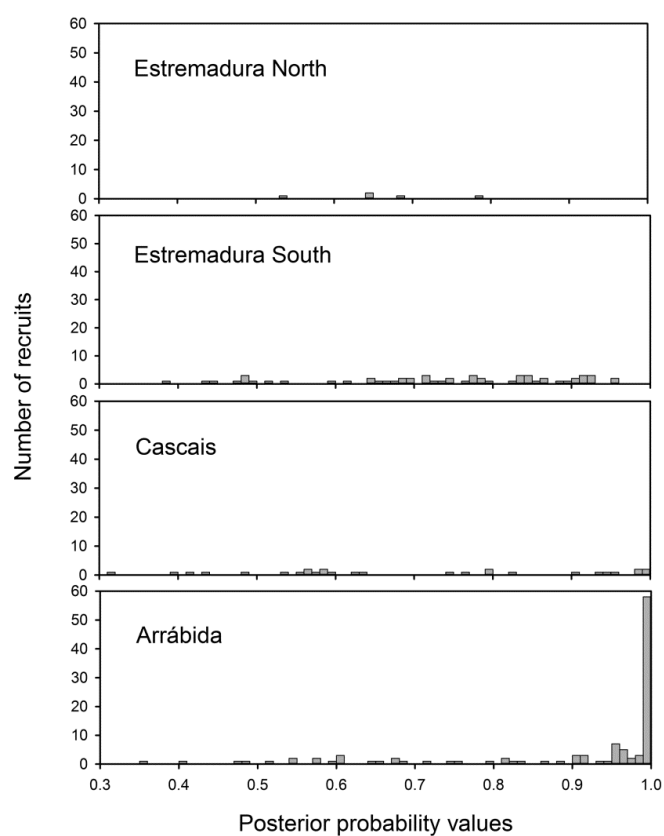


Figure 4.4 Posterior probabilities of assignment of mussel recruits into four putative origins, based on linear discriminant functions trained with larval shell elemental profiles.

To compare connectivity matrices estimated by the two methods (geochemical fingerprint vs biophysical model), we based our analysis first on the Observed and Modelled connectivity matrices uncorrected for unknowns, and then on matrices corrected for both Type 2 and Type 3 recruits (Table 4.2). We did this because the elemental fingerprinting technique and the DFA cannot distinguish between the two sources of uncertainty, and therefore comparisons based on each correction separately are uninformative. However, we provide the full set of comparisons in the Supplementary Information 3-Matrix correlations.

From the set of comparisons without correcting for unknowns, the best correlations correspond to the 3x3 spatial grids, reaching correlation coefficients over 0.90 for several scenarios of spawning and larval behaviour (Table 4.2). However, when larvae were forced to dwell in the bottom layer (Bl) the correlations decreased dramatically (to an average of 0.44 correlation). The 3x3 spatial grid scenarios that incorporated passive (Pa) or ontogenetic behaviours (Om), and simulated reproductive exhaustion (progressive decline in larval emission towards the end of the study period, S3 and S4), produced high correlation coefficients between the two matrices. This was particularly true (average 0.93 correlation) when no larvae were released from July onwards (S4). Using spatial grids with higher spatial resolution (3x4 and 4x4 matrixes) caused the correlations to drop progressively, although they were still elevated ( $r > 0.70$ ) in some scenarios. This reduction is related to a decrease in accuracy of recruit assignment based on shell geochemistry, as the signatures are not distinct at this spatial resolution (DFA reclassification success for the larvae in Gomes et al. (2016)). In both 3x3 and 4x4 arrangements, recruits predicted by the biophysical model to settle in the Estremadura (north and south) region showed the worst fit to the observations, but the model was well fitted to describe natal origins for recruits which settle in the Arrábida Bay, and to a lesser degree in the Cascais Bay (Supplementary Information 4-Matrix adjustment). When we changed the APT (Table 4.2), we obtained a similar pattern for most combinations, where best model fits correspond to thresholds around 0.75-0.95. The less restrictive scenario (APT better-than-the-rest and 0.50) showed the lowest correlation between Observed and Modelled matrices, with the exception of the 4x4 core matrices scenarios. For APTs of 0.75 to 0.99, correlations were quite similar for most of the scenarios, and maxima often fell around 0.90. That seems to indicate that the model reproduces the observed data when we maintain a moderate to high threshold, except for the 4x4 scenarios where the uncertainty of the geochemical data is higher.

Table 4.2 Pearson correlation coefficients between Observed and Modelled connectivity matrices for different combinations of larval behaviour, spawning regime, partitioning of the core region, and confidence level of the assignment of recruits into source populations. The top section refers to the core matrices without correction for unknowns; the bottom section refers to the core matrices plus unknown's row, where the modelled matrix was corrected for Type 2 and Type 3 recruits simultaneously. Shading indicates strength of the correlation; bold values indicate the highest correlation in each section.



CORE MATRICES (without unknown row); uncorrected modelled matrix		Larval behaviour / Spawning regime combinations											
		PaS1	PaS2	PaS3	PaS4	OmS1	OmS2	OmS3	OmS4	BIS1	BIS2	BIS3	BIS4
Partitioning of the core region / Confidence level combinations	3x3%99	0.64	0.72	0.77	0.87	0.58	0.67	0.75	0.82	0.28	0.31	0.34	0.38
	3x3%95	0.77	0.84	0.88	0.94	0.71	0.79	0.86	0.90	0.41	0.44	0.47	0.51
	3x3%90	0.77	0.84	0.88	0.94	0.72	0.80	0.86	0.91	0.41	0.44	0.47	0.50
	3x3%75	0.80	0.86	0.90	0.96	0.74	0.82	0.88	0.92	0.45	0.49	0.52	0.55
	3x3%50	0.79	0.85	0.89	0.94	0.72	0.80	0.87	0.91	0.41	0.44	0.47	0.51
	3x3 Better	0.77	0.83	0.88	0.93	0.70	0.78	0.85	0.89	0.38	0.42	0.45	0.49
	3x4%99	0.41	0.48	0.53	0.63	0.40	0.49	0.56	0.61	0.15	0.17	0.19	0.22
	3x4%95	0.61	0.67	0.71	0.79	0.59	0.67	0.73	0.78	0.33	0.36	0.38	0.41
	3x4%90	0.62	0.69	0.72	0.80	0.60	0.68	0.75	0.79	0.34	0.37	0.39	0.41
	3x4%75	0.62	0.68	0.72	0.79	0.61	0.69	0.74	0.77	0.37	0.39	0.42	0.44
	3x4%50	0.59	0.65	0.69	0.75	0.58	0.66	0.72	0.74	0.32	0.35	0.37	0.40
	3x4 Better	0.56	0.62	0.66	0.72	0.55	0.63	0.69	0.71	0.29	0.31	0.34	0.36
	4x4%99	0.40	0.47	0.50	0.60	0.38	0.46	0.52	0.56	0.13	0.15	0.17	0.19
	4x4%95	0.42	0.49	0.53	0.61	0.41	0.49	0.55	0.58	0.16	0.18	0.20	0.22
	4x4%90	0.52	0.58	0.61	0.68	0.51	0.57	0.62	0.63	0.25	0.26	0.29	0.29
	4x4%75	0.63	0.68	0.71	0.76	0.61	0.66	0.70	0.68	0.35	0.36	0.38	0.37
	4x4%50	0.66	0.71	0.73	0.78	0.64	0.69	0.73	0.70	0.38	0.38	0.41	0.40
	4x4 Better	0.65	0.70	0.72	0.76	0.64	0.69	0.72	0.69	0.37	0.38	0.40	0.38

**Pa**= passive larvae. **Om**= larvae migrating ontogenetically. **Bl**= larvae dwelling in the bottom layer. **S1**= continuous larval emission during each high tide until July 12. **S2**= continuous larval emission during each high tide until June 30, then larval emission skipping one of every two high tides until July 12. **S3**= continuous larval emission during each high tide until June 30, then larval emission skipping two of every three high tides until July 12. **S4**= Continuous larval emission during each high tide until July 1, no more larvae released afterwards. **3x3, 3x4 and 4x4**= spatial organization of the core region into 3 or 4 origin x destination cells. **Better than the rest**= recruits assigned into an origin when the probability of pertaining to that origin is better than that of pertaining to any other origin. **%99, %95, %90, %75, %50**= recruits assigned into an origin when the probability of pertaining to that origin is larger than the level indicated.

CORE MATRICES + UNKNOWN; modelled matrix corrected for Type 2 and Type 3 recruits		Larval behaviour / Spawning regime combinations											
		PaS1	PaS2	PaS3	PaS4	OmS1	OmS2	OmS3	OmS4	BIS1	BIS2	BIS3	BIS4
Partitioning of the core region / Confidence level combinations	3x3%99	0.47	0.55	0.61	0.76	0.51	0.58	0.62	0.71	0.07	0.09	0.11	0.14
	3x3%95	0.63	0.71	0.77	0.88	0.65	0.72	0.76	0.83	0.20	0.23	0.25	0.28
	3x3%90	0.69	0.77	0.83	0.93	0.71	0.78	0.83	0.88	0.26	0.29	0.32	0.35
	3x3%75	0.70	0.77	0.82	0.88	0.70	0.77	0.82	0.83	0.36	0.39	0.42	0.45
	3x3%50	0.57	0.63	0.66	0.66	0.55	0.61	0.65	0.63	0.35	0.37	0.39	0.41
	3x3 Better	0.53	0.58	0.62	0.61	0.50	0.56	0.60	0.57	0.33	0.35	0.38	0.40
	3x4%99	0.55	0.60	0.63	0.73	0.52	0.57	0.61	0.67	-0.06	-0.04	-0.03	-0.02
	3x4%95	0.63	0.70	0.74	0.83	0.60	0.66	0.71	0.76	0.08	0.10	0.11	0.13
	3x4%90	0.63	0.70	0.74	0.83	0.59	0.66	0.72	0.78	0.16	0.18	0.20	0.22
	3x4%75	0.56	0.63	0.67	0.73	0.54	0.61	0.67	0.70	0.28	0.30	0.31	0.33
	3x4%50	0.32	0.37	0.39	0.42	0.31	0.36	0.41	0.42	0.30	0.31	0.33	0.34
	3x4 Better	0.25	0.30	0.32	0.35	0.24	0.30	0.34	0.35	0.28	0.29	0.31	0.32
	4x4%99	0.89	0.90	0.90	0.92	0.84	0.85	0.86	0.86	0.47	0.46	0.48	0.43
	4x4%95	0.89	0.92	0.93	0.96	0.86	0.88	0.90	0.92	0.48	0.47	0.49	0.45
	4x4%90	0.86	0.90	0.91	0.95	0.83	0.86	0.89	0.91	0.47	0.46	0.49	0.45
	4x4%75	0.75	0.79	0.82	0.86	0.73	0.78	0.81	0.83	0.45	0.44	0.47	0.43
	4x4%50	0.33	0.38	0.41	0.46	0.32	0.38	0.43	0.45	0.22	0.23	0.25	0.25
	4x4 Better	0.21	0.26	0.29	0.34	0.21	0.27	0.32	0.34	0.14	0.15	0.17	0.17

**Pa**= passive larvae. **Om**= larvae migrating ontogenetically. **BI**= larvae dwelling in the bottom layer. **S1**= continuous larval emission during each high tide until July 12. **S2**= continuous larval emission during each high tide until June 30, then larval emission skipping one of every two high tides until July 12. **S3**= continuous larval emission during each high tide until June 30, then larval emission skipping two of every three high tides until July 12. **S4**= Continuous larval emission during each high tide until July 1, no more larvae released afterwards. **3x3, 3x4 and 4x4**= spatial organization of the core region into 3 or 4 origin x destination cells. **Better than the rest**= recruits assigned into an origin when the probability of pertaining to that origin is better than that of pertaining to any other origin. **%99, %95, %90, %75, %50**= recruits assigned into an origin when the probability of pertaining to that origin is larger than the level indicated.

If we now take into consideration the recruits of unknown origin (Type 2 and Type 3, i.e. all the ones that failed to be successfully classified to one of the possible origins; Table 4.2) a different picture emerges. The contrasts between larval behaviours and spawning regimes still followed the same patterns as in the preceding case, but now the effect of increasing spatial resolution differs. When we corrected for Type 2 and Type 3 (Table 4.2) the correlations increased considerably in the higher spatial resolution scenarios. This effect is related to the increase in the number of unknowns in the geochemical classification with increasing spatial resolution, resulting in an improved fit between the observed and predicted recruits in the 4x4 grid, especially in the Estremadura region (Supplementary Information 4-Matrix adjustment). It is interesting to note that the biophysical model very accurately described natal origins for the Arrábida recruits as well, followed by those that recruited into Cascais. Again, we observed the same pattern as before, with higher correlations with APTs between 0.75 and 0.95 (Table 4.2).

#### **4.3.2 Assessing the causes of convergence between observed and modelled connectivity matrices**

Independently of whether we consider only the core connectivity matrices, or the connectivity matrices with an unknown row (origin), increasing the APTs increased matrix correlations up to a point between 0.75 and 0.95, after which matrix correlations decreased again (Table 4.2). Given this pattern, we make two predictions. The first prediction is that this effect is different from a random deletion of recruits from the Observed matrix. Increasing the confidence level from the "better-than-the-rest" case is akin to removing outliers from the Observed matrix, so we should expect that removing recruits at random would not result in an increased correlation. On the other hand, by being too strict in the assignment of recruits we could be removing individuals from the Observed matrix that are correctly classified, resulting in a decreased correlation. The second prediction is that the number of excluded recruits that provides the best correlation should logically match the number of those with a poorly defined elemental signature, plus those that originate from outside the core region. The first case reflects the compounded effect of assigning recruits to an unknown origin based on the misclassification rate of the larvae into their source population (i.e. the proportion of larvae incorrectly self-assigned in each region), which is a measure of the inherent variability of the elemental signature. That number is the number of Type 2 recruits that are assigned to the unknown row in the Modelled connectivity matrix. The second case is the number of Type 3 recruits.

We tested both predictions only for the core connectivity matrices, and for the connectivity matrices with an unknown row composed of Type 2 and Type 3 recruits, for the combination of continuous larval emission during each high tide until July 1 (S4) and passive larvae (Pa), which were the best biological scenarios overall, and for all spatial arrangements of the core matrix (3x3, 3x4 and 4x4). We used a bootstrap approach in order to test the first prediction. For each APT (0.50, 0.75, 0.90, 0.95 and 0.99) we generated 1000 Observed connectivity matrices by randomly discarding, from the better-than-the-rest matrix, a number of recruits equal to the sum of the worst classified recruits into every source. Each of the 1000 randomly adjusted Observed matrices for a given confidence level was then correlated with the corresponding Modelled matrix, and the frequency distribution of the correlation coefficients was generated. The correlation coefficient obtained from the comparison between the Observed matrix correctly adjusted for the confidence level and the Modelled matrix was then compared to that frequency distribution. To test the second prediction, we calculated the difference between the proportion of recruits classified as unknowns in the Observed matrix for each confidence level and the proportion of modelled unknowns, and plotted the correlation coefficient against this quantity.

In 19 cases out of 30 comparisons, the improvement of the matrix correlation obtained by increasing the APT was significantly different from that obtained by a random deletion of recruits from the Observed matrix (Supplementary Information 5-Prediction 1). The cases where the improvement was most significant corresponded to the 4x4 spatial arrangement (Fig. 4.5,  $p < 0.0001$  for the 0.75, 0.90, 0.95 and 0.99 APTs), which included also the highest correlation coefficient obtained across all scenarios ( $r = 0.96$ , Table 4.2, passive larvae (Pa), cessation of spawning after July (S4), 0.95 APT, 4x4 spatial arrangement). In the case of the 3x3 and 3x4 spatial arrangements the matrix correlation peaked when the difference between observed and modelled unknown recruits approached zero (at an APT of 0.90), and at a slightly positive value in the case of the 4x4 arrangement (at an APT of 0.95; Fig. 4.6).

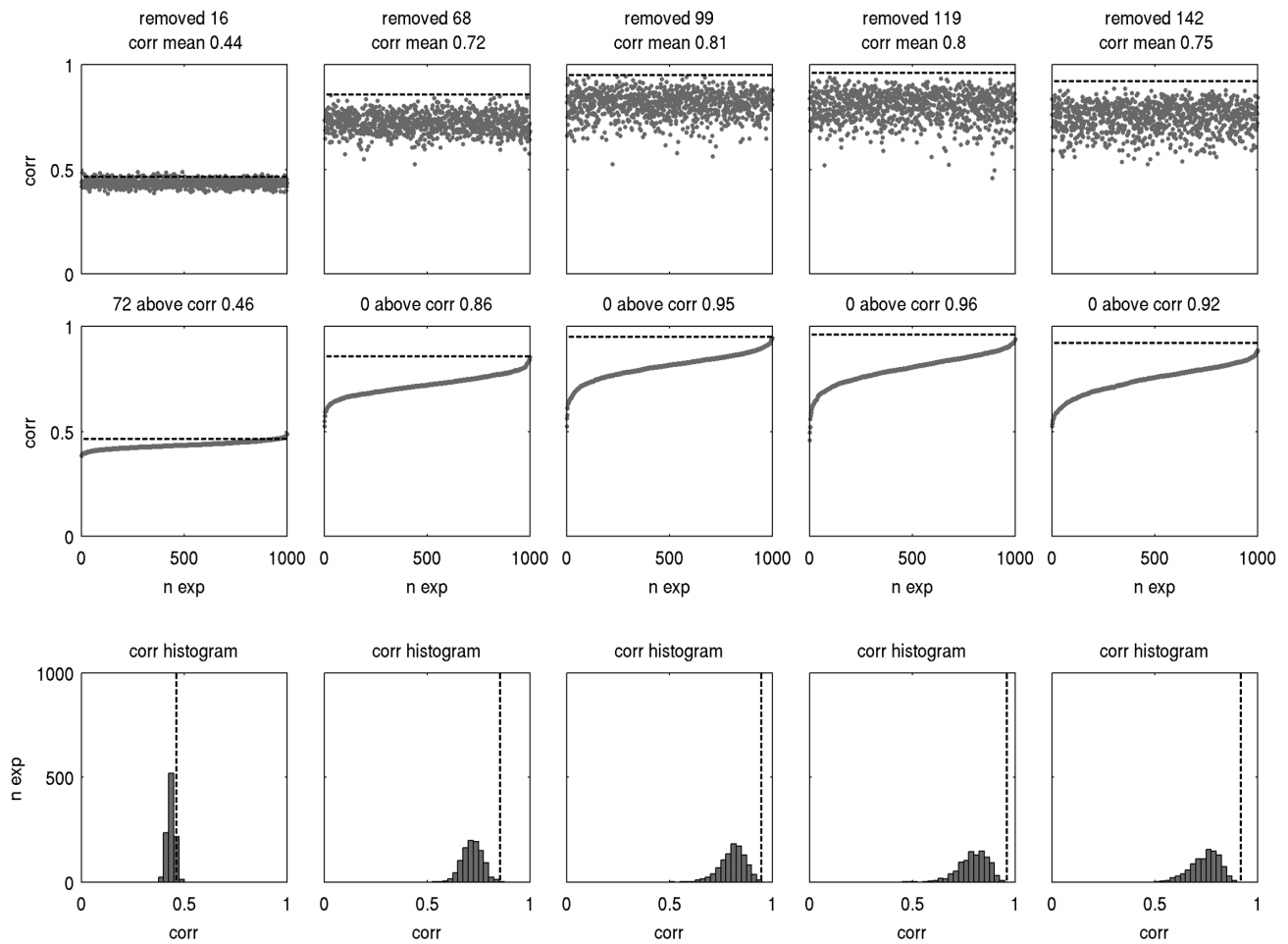


Figure 4.5 Effect on the matrix correlation coefficient of randomly excluding from the observed matrix a number of individuals equal to the number of observed individuals correctly classified as unknowns for each confidence level (columns are Assignment Probability Thresholds (APTs) of 0.50, 0.75, 0.90, 0.95 and 0.99), based on 1000 trials for each threshold. In each graph, the dashed line indicates the correlation coefficient that was obtained by removing those recruits that correctly failed to pass the APT. First row: distribution of correlation coefficients by trial number; the number of removed individuals is indicated above each graph. Second row: the same, but correlation coefficients ranked by value; the number of trials with a correlation coefficient above that obtained by removing those recruits that correctly failed to pass the posterior probability threshold is indicated above each graph. Third row: frequency distribution of the correlation coefficients. Removing the recruits that correctly failed to pass the APT resulted in a correlation coefficient significantly higher than that obtained by a random deletion of recruits at  $p < 0.0001$  (\*\*\*\*). "corr"= correlation coefficient. The figure only shows results for the 4x4 arrangement, passive larvae and the S4 spawning scenario (see Supplementary Information 5-Prediction 1 for other scenarios).

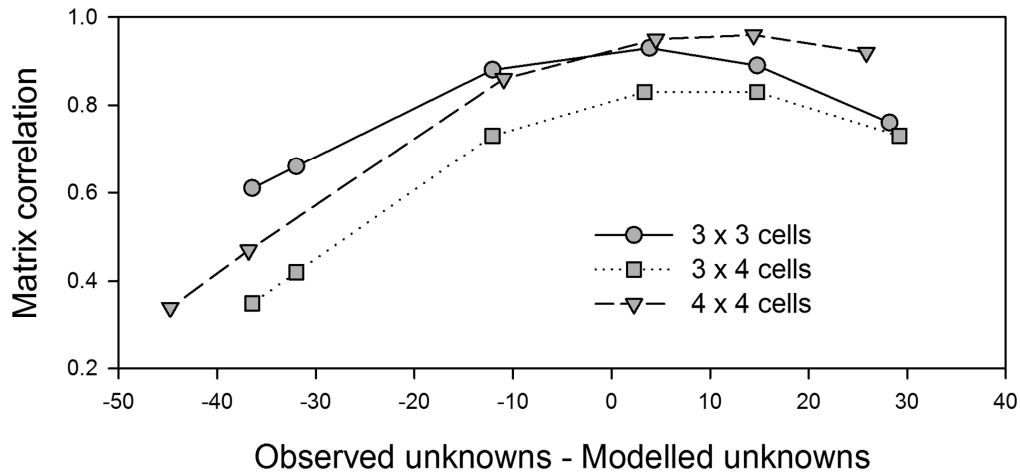


Figure 4.6 Relationship between the matrix correlation coefficient and the difference between the numbers of observed and modelled recruits classified as unknowns, for three different arrangements of the connectivity matrices. The number of observed recruits classified as unknowns changes with the threshold level (from left to right, APTs better-than-the-rest, then above 0.50, 0.75, 0.90, 0.95 and 0.99). The number of modelled recruits classified as unknowns depends on the misclassification rate of the larvae into their source population (proportion of larvae incorrectly self-assigned in each region; Type 2 recruits) and on those that originate from outside the core region (Type 3 recruits). The figure only shows results for passive larvae and the S4 spawning scenario (see Supplementary Information 6-Prediction 2 for other scenarios).

Visual inspection of Observed and Modelled connectivity matrices for the 0.95 APT (Table 4.3; other thresholds not shown, but very similar results were obtained for 0.90; see also Supplementary Information 6-Prediction 2 for Type 2 and Type 3 recruits) indicates that the poorest performance of the model relative to the observations occurred in the Estremadura region. This is especially evident in the case of the 4x4 arrangement, where observations indicate supply to Estremadura S and Estremadura N from the southern regions, while the model indicates higher retention or supply from the north, especially in Estremadura N. Both observations and model results concur in identifying the Arrábida Bay as a region of high retention but also as a major supplier to Cascais and Estremadura S.

Table 4.3 Observed and Modelled connectivity matrices obtained for the scenarios of passive larvae and cessation of spawning after July, for the 3x3, 3x4 and 4x4 spatial arrangements. The top six panels refer to the core matrices without correction for unknowns and the better-than-the-rest assignment probability threshold; the bottom six panels refer to the core matrices plus unknown's row for a 0.95 assignment probability threshold, where the Modelled matrix was corrected for Type 2 and Type 3 recruits simultaneously. Shading indicates the strength of connectivity.

**Core connectivity matrices (without correction for unknowns)**

**Observed, better-than-the-rest case**

Destination Origin	Estremadura	Cascais	Arrábida
Estremadura	34	17	9
Cascais	24	7	16
Arrábida	41	77	74

Destination Origin	Estremadura N	Estremadura S	Cascais	Arrábida
Estremadura	33	35	17	7
Cascais	43	7	7	19
Arrábida	23	57	77	74

Destination Origin	Estremadura N	Estremadura S	Cascais	Arrábida
Estremadura N	7	1	0	0
Estremadura S	37	35	17	14
Cascais	33	4	3	9
Arrábida	23	59	80	77

**Modelled, without correction for Type 2 or Type 3 recruits**

Destination Origin	Estremadura	Cascais	Arrábida
Estremadura	58	9	0
Cascais	5	13	1
Arrábida	37	78	99

Destination Origin	Estremadura N	Estremadura S	Cascais	Arrábida
Estremadura	100	52	9	0
Cascais	0	6	13	1
Arrábida	0	42	78	99

Destination Origin	Estremadura N	Estremadura S	Cascais	Arrábida
Estremadura N	67	4	0	0
Estremadura S	33	48	9	0
Cascais	0	6	13	1
Arrábida	0	42	78	99

**Connectivity matrices with unknown row****Observed, 0.95 assignment probability threshold**

Destination Origin	Estremadura	Cascais	Arrábida
Estremadura	14	7	0
Cascais	6	0	2
Arrábida	23	63	49
Unknown	57	30	49

Destination Origin	Estremadura N	Estremadura S	Cascais	Arrábida
Estremadura	17	12	7	0
Cascais	13	0	0	2
Arrábida	5	38	63	49
Unknown	65	50	30	49

Destination Origin	Estremadura N	Estremadura S	Cascais	Arrábida
Estremadura N	0	0	0	0
Estremadura S	0	3	0	0
Cascais	8	0	0	0
Arrábida	7	43	67	51
Unknown	85	54	33	49

**Modelled, corrected for Type 2 and Type 3 recruits**

Destination Origin	Estremadura	Cascais	Arrábida
Estremadura	39	8	0
Cascais	2	8	0
Arrábida	21	59	61
Unknown	38	25	39

Destination Origin	Estremadura N	Estremadura S	Cascais	Arrábida
Estremadura	22	47	8	0
Cascais	0	4	8	0
Arrábida	0	31	59	61
Unknown	78	18	25	39

Destination Origin	Estremadura N	Estremadura S	Cascais	Arrábida
Estremadura N	13	4	0	0
Estremadura S	3	19	4	0
Cascais	0	4	8	0
Arrábida	0	31	59	61
Unknown	84	42	30	39



## 4.4 Discussion

### 4.4.1 Comparison between observed and modelled connectivity matrices

In the present study we manipulated an Observed connectivity matrix, derived from geochemical information of mussel larval and recruit shells, by applying different assignment probability thresholds (APTs) to the classification of recruits into the source populations based on the posterior probabilities of assignment. Recruits that failed to pass the prescribed APT were assigned to an unknown category. We also manipulated a Modelled connectivity matrix derived from a biophysical model by using different population and larval biology scenarios. Moreover, we simulated the intrinsic variability of the geochemical signal by classifying modelled recruits as unknowns in a proportion equivalent to the misclassification rate of the larvae to their own sources, which is a measure of the inherent variability of the elemental profile. A second source of uncertainty was addressed by also classifying as unknowns the modelled recruits that originated outside the region for which elemental data were available. We obtained a very good convergence between the two methods at the lowest spatial resolution when no correction for unknowns was applied, with correlation coefficients  $r$  up to 0.96, but a worse fit at the highest spatial resolution with  $r < 0.76$ . When we corrected for unknowns the convergence between the two methods at the higher spatial resolution increased substantially to values of  $r > 0.80$  and up to 0.93 and 0.96, for APTs between 0.90 and 0.95, passive or ontogenetically migrating larvae, and realistic spawning scenarios. As far as we know, there is no precedent for this level of convergence between two independent estimates of larval dispersal and connectivity at spatial scales below 40 km.

The interpretation of the fit between the two approaches requires a phenomenological interpretation of the dispersal process captured during this event (Gomes et al. 2016). The geochemical signatures indicated an overall northward dispersal of larvae, with those originating in the Arrábida Bay contributing disproportionately to the Cascais Bay and the Estremadura regions. This northward dispersal event runs contrary to the average circulation along the Portuguese coast during spring and summer, associated with upwelling circulation (Relvas et al. 2007), but is consistent with concurrent wind data that shows a 3-week long upwelling relaxation episode that took place just prior to the sampling of the recruits (Gomes et al. 2016). The relaxation episode was accompanied by a distinct temperature increase caused by the northward advection of warm waters, which was well captured by the biophysical model (Supplementary Information 2-Biophysical model). The high correlation coefficients obtained with a 3x3 spatial arrangement of the core zone, with

passive and ontogenetic larval behaviour scenarios, are a consequence of the small spatial resolution overall (about 20, 30 and 70 km in the Cascais, Arrábida and Estremadura regions, respectively). As we increased spatial resolution by subdividing the Estremadura region we decreased the ability to assign recruits to their source populations based on the natal signatures, as the spatial resolution fails to be adequate to achieve good geospatial distinct chemical signals. However, when we incorporated the unknowns into a virtual box, both in the Observed and in the Modelled matrices, there still was a high correlation coefficient ( $r > 0.80$ ) for a large range of biological scenarios and APTs, reaching a maximum of 0.96. That is, by explicitly modelling the uncertainty sources of the elemental fingerprinting technique, we were able to simultaneously increase the overall spatial resolution of the analysis (20, 30, 40, 30 km, for the Cascais, Arrábida, Estremadura south and Estremadura north regions, respectively) and the fit of the model to the observations.

#### **4.4.2 Assessing the causes of convergence between observed and modelled connectivity matrices**

The numerical changes in the correlation coefficient with the shifting APTs were not due to random effects, with maximum correlations occurring when number of observed unknowns approached modelled unknowns, or slightly exceeded them in the case of the 4x4 spatial scenario. This last result suggests that the model underestimates the contribution of Type 3 recruits, or that the correction for Type 2 recruits has been overestimated, which could result from a small sample of the posterior probabilities as spatial resolution was increased. Other discrepancies between the observations and the model were the poor match in the Estremadura region. These discrepancies may arise from limitations of the elemental fingerprinting technique and of the model. Elemental fingerprinting requires that sufficient chemical variability of the water be present over space, but also that the chemistry of the calcified structures in some way reflects the physicochemical properties of the water. Controlled laboratory experiments indicate linear relationships between the concentrations of several elements in seawater and in calcified structures (mollusc larval shells and statoliths (Milton and Chenery 2001, Zacherl et al. 2003), but also interactive effects of temperature and salinity (fish otoliths Martin and Thorrold (2005); mollusc larval shells Andreia Carvalho and Laura Peteiro, unpublished data) that will influence the multivariate distribution of elements in the target structure and may complicate the probabilistic assignment of individuals and the interpretation of the patterns. The biophysical model on the other hand is constrained by its internal variability and may not be resolving

appropriately all details of the oceanography and biology. For instance, although the model configuration is designed to solve the continental shelf circulation at the scale of the Western Iberian Margin, the inner continental shelf circulation is influenced by local cross-shelf winds and surface gravity waves (not solved), and is characterized by a logarithmic shoreward decrease in current velocity at scales of 1-2 km (Nickols et al. 2012), which likely affect the estimates of along-shore transport. Additionally, although we have obtained consistent estimates of dispersal across a range of spawning and of larval behaviour scenarios (except in the case of unrealistic bottom-dwelling larvae (Mcquaid and Phillips 2000, Fuchs and DiBacco 2011), we used growth and mortality rates derived from the literature (Ruiz et al. 2008, Pettersen et al. 2010) without a formal assessment of the model sensitivity to their variability.

#### 4.5 Future directions

In the present study we obtained high correlations ( $r = 0.96$ ) between Observed and Modeled connectivity matrices obtained by both approaches at a high spatial resolution (20 - 40 km), after discarding all recruits that failed to pass a stringent assignment probability threshold (APT = 0.95), in spite of other internal sources of error inherent to either methodology. Most of these recruits originated from the Arrábida Bay, which is distinguished from the other sources by a well-defined elemental signature. An argument can be drawn that, if the model describes these larvae with high certainty, it should also be well fitted to predict mussel larvae dispersal and trajectories in the remaining central Portuguese west coast. We propose that targeting dispersing individuals for which we have of high certainty of assignment to a natal population is an effective way of validating biophysical models of larval dispersal, allowing stronger inferences on population connectivity relevant for the management of marine populations. Presently, the demonstration of the biophysical model accuracy at smaller spatial scales seems to be limited by the resolution of the geochemical fingerprinting technique. The approach taken here also highlights the potential in using these two techniques in an integrated manner, in order to compensate for, and explore, different spatial resolutions and sources of uncertainty (Mora and Sale 2002, Thorrold et al. 2002, Levin 2006, Werner et al. 2007, Thorrold et al. 2007, Cowen and Sponaugle 2009, Burgess et al. 2014), and opens the door to effectively combine the two techniques to investigate the ability of biophysical models *per se* to describe a wider range of biological models, geographical settings and temporal scales.

## 4.6 References

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#### 4.7 Supplementary Information 1: Literature review

##### Analysis

The two most common approaches have been to use genetic markers and a numerical biophysical model, or the microchemistry of hard parts and a numerical biophysical model, but genetic markers and micro-chemistry, and combinations of genetic markers or microchemistry with current measurements, have also been employed. Ideally, a migration probability matrix could be produced by each methodology, which would allow a spatially explicit estimate of intensity of dispersal and connectivity, and a direct numerical comparison of dispersal estimates. However, constraints associated with life-cycle traits, type of biological material and, presumably, available expertise and funding resulted in a variety of dispersal metrics employed by the different studies, which necessarily influenced the types of comparisons that could be made between estimates.

Genetic techniques most commonly employed estimated distance matrices among sampled populations based on a variety of indices ( $F_{ST}$ ,  $G_{ST}$ , Jost's  $D_{est}$ , Nei's  $D_A$ ) derived from microsatellites (Galindo et al. 2006, Baums et al. 2006, Dupont et al. 2007, Jolly et al. 2009, Salas et al. 2009, Liu et al. 2010, White, Selkoe et al. 2010, Kool et al. 2010, Kool et al. 2011, Alberto et al. 2011, Foster et al. 2012, Berry et al. 2012a, Coscia et al. 2012, Di Franco et al. 2012, Thomas and Bell 2013, Jolly et al. 2013, Sunday et al. 2014, Davies et al. 2014, Young et al. 2015, Thomas et al. 2015, Gormley et al. 2015), COI (Galindo et al. 2010, Crandall et al. 2012), cytochrome b (Chiswell 2009), the control region (Correia et al. 2012, Ben-Tzvi et al. 2012), or elongation factor 1 alfa (Galindo et al. 2010), for a total of thirty three studies. Two studies calculated a multi-generation migration probability matrix using coalescent analysis of gene flow based on microsatellites (Jolly et al. 2009) or on COI (Crandall et al. 2012). Nine studies estimated a contemporary migration probability matrix using genetic (Miller et al. 2005, Bradbury et al. 2008, Alberto et al. 2011, Schunter et al. 2011, Thomas and Bell 2013, Simpson et al. 2014, Fraker et al. 2015) or parentage assignment tests (Berumen et al. 2010, Nanninga et al. 2015), all based on microsatellites.

Studies using the microchemistry of hard parts (elemental fingerprinting) have been less commonly applied in conjunction with other approaches, likely because the technique can be applied to a much smaller range of organisms (typically fishes and bivalves). We identified only four studies that calculated a contemporary migration probability matrix (Miller et al. 2005, Liu et al. 2010, Cook et al. 2014, Fraker et al. 2015), all applying discriminant function analysis to assign individuals to putative source populations. Four other studies used

elemental fingerprinting to clarify similarities among cohorts (Ben-Tzvi et al. 2012), classify self-recruits *versus* dispersers (Berumen et al. 2010) and freshwater dispersers *versus* marine dispersers (Bradbury et al. 2008), or to characterize source locations (Correia et al. 2012).

Biophysical numerical modelling was the technique most commonly used in tandem with a second (or third) approach to investigate connectivity (35 out of the 40 studies). Biophysical models coupled a baroclinic hydrodynamic model adjusted to local bathymetry, with a biological model describing spawning distribution and larval biology. The oceanographic models differed in spatial scale, nesting, grid resolution and forcing. In all cases different combinations of spawning periodicity and intensity, growth rate, mortality rate, competency period and/or larval behaviour were employed, in an attempt to bracket the uncertainty regarding parameterization of these processes. Simulated periods were chosen to cover the expected sources of temporal variability, whereas some kind of temporal integration was typically applied in order to calculate an average connectivity matrix. Twenty one studies estimated dispersal probability matrices (Galindo et al. 2006, Baums et al. 2006, Dupont et al. 2007, Piggott et al. 2008, Chiswell 2009, Jolly et al. 2009, Salas et al. 2009, Galindo et al. 2010, Alberto et al. 2011, Schunter et al. 2011, Berry et al. 2012, Soria et al. 2012, Coscia et al. 2012, Crandall et al. 2012, Di Franco et al. 2012, Thomas and Bell 2013, Simpson et al. 2014, Schiavina et al. 2014, Gormley et al. 2015), while fourteen studies calculated migration probability matrices (White et al. 2010, Kool et al. 2010, 2011, Selkoe et al. 2010, Foster et al. 2012, Domingues et al. 2012, Jolly et al. 2013, Moksnes et al. 2014, Sunday et al. 2014, Davies et al. 2014, Nanninga et al. 2015, Fraker et al. 2015, Young et al. 2015, Thomas et al. 2015).

Advancements on the merging of independent approaches to describe dispersal patterns have been to use connectivity matrices predicted by biophysical models (either dispersal probability matrices or migration probability matrices) into population genetic models, in order to predict genetic structure. If the predicted genetic structure matches the observed structure, a case is made that migration mediated by oceanographic patterns of propagule transport influences gene flow. Five studies used a derivation of the Bodmer and Cavalli-Sforza matrix model of migration (Bodmer and Cavalli-Sforza 1968) to predict equilibrium allele frequencies after a variable number of generations, and compared these with observations through correlation between distance matrices (Foster et al. 2012, Young et al. 2015), comparison between isolation-by-distance and isolation-by-oceanographic distance statistics tested separately (White et al. 2010), or qualitatively (Kool et al. 2010, 2011). Similar approaches, where modelled pairwise migration probabilities were used to inform a

population model predicting allele frequencies at equilibrium, were applied by another three studies, which used Mantel tests to compare matrices of observed and predicted genetic distances (Sunday et al. 2014), sums of squared differences between predicted and observed allele frequencies (Galindo et al. 2010), or qualitative assessments of fit (Galindo et al. 2006) to compare predicted and observed genetic structure.

Supplementary Table 4.1.1. Methodologies employed by studies that used a multiple approach to the estimation of marine larval dispersal and connectivity matrices.

Approaches	Study taxa	Location	Dispersal metrics	Uncertainty	Type of comparison	Reference
Genetics and Model	Giant kelp, <i>Macrocystis pyrifera</i>	Santa Barbara Channel, California	Genetic distance matrix; migration probability matrix calculated using genetic assignment; modeled dispersal probability matrix used to estimate probability-weighted mean transport time	Not accounted	Semi-quantitative; comparison of the percentage variability explained by trends of isolation-by-distance and isolation-by-oceanographic distance	(Alberto et al. 2011)
Genetics and Model	Coral, <i>Acropora palmata</i>	Caribbean	Genetic distance matrix; modeled dispersal probability matrix	Not accounted	Qualitative	(Baums et al. 2006)
Genetics and Model	Coral reef fish, <i>Glaucosoma hebraicum</i>	Western Australia	Genetic distance matrix; modeled dispersal probability matrix; modeled transport probability	Not accounted	Quantitative; Mantel test used to compare matrices of observed and predicted distances	(Berry et al. 2012a)
Genetics and Model	Coral reef fish, <i>Lethrinus nebulosus</i>	Northwestern Australia	Genetic distance matrix; modeled dispersal probability matrix	Not accounted	Qualitative	(Berry, et al. 2012b)
Genetics and Model	Common cockle, <i>Cerastoderma edule</i>	Southern Irish Sea	Genetic distance matrix; modeled dispersal probability matrix;	Not accounted	Qualitative	(Coscia et al. 2012)
Genetics <sup>a</sup> and Model	Marine gastropods, <i>Nerita plicata</i> and <i>Nerita albicilla</i> ; amphidromous gastropods <i>Neritina canalis</i> and <i>Neritina dilatatum</i>	Indo-Pacific	Coalescent analysis of gene flow fed with modeled dispersal probability matrix to predict observed genetic structure	Not accounted	Quantitative; log Bayes factors analysis that the predicted genetic structure fits the observed genetic structure	(Crandall et al. 2012)
Genetics and Model	Corals, <i>Acropora hyacinthus</i> and <i>A. digitifera</i>	Greater Micronesia	Genetic distance matrix; modeled migration probability matrix transformed into oceanographic distance	Not accounted	Semi-quantitative; comparison of the percentage variability explained by trends of isolation-by-distance and isolation-by-oceanographic distance	(Davies et al. 2014)
Genetics and	White sea bream,	Apulian	Genetic distance matrix; modeled	Not accounted	Qualitative	(Di Franco et

Approaches	Study taxa	Location	Dispersal metrics	Uncertainty	Type of comparison	Reference
Model	<i>Diplodus sargus sargus</i>	coast, Adriatic	dispersal probability matrix			al. 2012)
Genetics and Model	Shore crab, <i>Carcinus maenas</i>	Western Iberian Peninsula	Genetic distance matrix; modeled migration probability matrix	Not accounted	Qualitative	(Domingues et al. 2012)
Genetics <sup>e</sup> and Model	Gastropod, <i>Crepidula fornicata</i>	English Channel	Genetic distance matrix; modeled dispersal probability matrix; modeled transport probability matrix	Not accounted	Quantitative; Mantel test used to compare observed and predicted distance matrices	(Dupont et al. 2007)
Genetics and Model	Coral, <i>Montastraea annularis</i>	Caribbean	Genetic distance matrix; modeled migration probability matrix used to project allele frequencies after 100 generations based on Bodmer and Cavalli-Sforza (1968) matrix model of migration	Not accounted	Quantitative; non-parametric correlation between distance matrices	(Foster et al. 2012)
Genetics and Model	Coral, <i>Acropora cervicornis</i>	Caribbean	Genetic distance matrix from previous study; modeled dispersal probability matrix fed into a population genetic model to simulate multi-generations genetic distance based on 10 independent loci	Not accounted	Qualitative	(Galindo et al. 2006)
Genetics <sup>a, d</sup> and Model	Barnacle, <i>Balanus glandula</i>	California	Observed allele frequencies; modeled migration probability matrix used to estimate deterministic transitions of allele frequencies after 200 generations	Not accounted	Quantitative; sum of squared differences between predicted and observed allele frequencies	(Galindo et al. 2010)
Genetics and Model	Horse mussel, <i>Modiolus modiolus</i>	Irish Sea	Genetic distance matrix; modeled dispersal probability matrix	Not accounted	Qualitative	(Gormley et al. 2015)
Genetics and Model	Polychaete, <i>Pectinaria koreni</i>	English Channel,	Coalescent analysis of gene flow; genetic distance matrix; modeled	Not accounted	Qualitative	(Jolly et al. 2009)

Approaches	Study taxa	Location	Dispersal metrics	Uncertainty	Type of comparison	Reference
		British Islands, North Sea	multi-generation dispersal probability matrix			
Genetics <sup>e</sup> and Model	Polychaete, <i>Pectinaria koreni</i>	Seine Bay	Genetic distance matrix; modeled migration probability matrix	Not accounted	Qualitative	(Jolly et al. 2013)
Genetics and Model	Model coral species	Caribbean	Genetic distance matrices from previous studies; modeled migration probability matrix used to project allele frequencies after 100 generations based on Bodmer and Cavalli-Sforza (1968) matrix model of migration	Not accounted	Qualitative	(Kool et al. 2010)
Genetics and Model	Model coral species	Indo-West Pacific	Genetic distance matrices from previous studies; modeled migration probability matrix used to project allele frequencies after 100 generations based on Bodmer and Cavalli-Sforza (1968) matrix model of migration	Not accounted	Qualitative	(Kool et al. 2011)
Genetics and Model	Shore crab, <i>Carcinus maenas</i>	North Sea, Kattegat, Skagerrak	Genetic distance matrices from previous studies; modeled dispersal single- generation and multiple-generation probability matrix	Not accounted	Qualitative	(Moksnes et al. 2014)
Genetics and Model	Anemonefish, <i>Amphiprion bicinctus</i>	Central Red Sea	Migration probability matrix calculated using parentage assignment; modeled self-retention and self-recruitment	Not accounted	Qualitative	(Nanninga et al. 2015)
Genetics and Model	Abalone, <i>Haliotis coccoradiata</i>	New South Wales, Australia	Genetic distance matrix; modeled dispersal probability matrix	Not accounted	Qualitative	(Piggott et al. 2008)



Approaches	Study taxa	Location	Dispersal metrics	Uncertainty	Type of comparison	Reference
Genetics and Model	Bicolor damselfish, <i>Stegastes partitus</i>	Central American Gulf coast	Genetic distance matrix; modeled dispersal probability matrix	Not accounted	Qualitative	(Salas et al. 2009)
Genetics and Model	Mediterranean shore crab, <i>Carcinus aestuarii</i>	Adriatic	Genetic distance matrix and Bayesian clustering; modeled dispersal probability matrix	Not accounted	Qualitative	(Schiavina et al. 2014)
Genetics and Model	Fish, <i>Serranus cabrilla</i>	Mediterranean	Migration probability matrix calculated using genetic assignment; modeled dispersal probability matrix	Accounted using a posterior probability threshold of 0.80 for correct assignment to parental population	Quantitative; correlation between connectivity matrices	(Schunter et al. 2011)
Genetics and Model	Clownfish, <i>Amphiprion omanensis</i>	Oman, Arabic Sea	Migration probability matrix calculated using genetic assignment; modeled dispersal probability matrix	Accounted using a posterior probability threshold of 0.95 for correct assignment to parental population	Qualitative	(Simpson et al. 2014)
Genetics and Model	Rock scallop, <i>Spondylus calcifer</i>	Northern Gulf of California	Genetic distance matrix; modeled dispersal probability matrix	Not accounted	Qualitative	(Soria et al. 2012)
Genetics and Model	Bat star, <i>Patiria miniata</i>	Northwestern Pacific	Genetic distance matrices from a previous study; modeled dispersal probability matrix fed into a genetic population model to simulate multi-generations genetic distance based on 50 independent loci	Not accounted	Quantitative; Mantel test used to compare matrices of observed and predicted genetic distances	(Sunday et al. 2014)
Genetics and Model	Rock lobster, <i>Jasus edwardsii</i>	Tasmanian Sea	Migration probability matrix calculated using genetic assignment; modeled dispersal probability matrix	Not accounted	Qualitative	(Thomas and Bell 2013)
Genetics and	Coral, <i>Acropora</i>	Houtman	Genetic distance matrix; modeled	Not accounted	Semi-quantitative; significance of	(Thomas et

Approaches	Study taxa	Location	Dispersal metrics	Uncertainty	Type of comparison	Reference
Model	<i>spicifera</i>	Abrolhos Islands, Western Australia	migration probability matrix projected forward for 10 generations used to calculate oceanographic distance		isolation-by-distance and isolation-by-oceanographic distance tested separately	al. 2015)
Genetics and Model	Whelk, <i>Kelletia kelletii</i>	California	Genetic distance matrix; modeled migration probability matrix used to project allele frequencies after 1000 generations based on Bodmer and Cavalli-Sforza (1968) matrix model of migration and used to estimate oceanographic distance	Not accounted	Semi-quantitative; significance of isolation-by-distance and isolation-by-oceanographic distance tested separately	(White et al. 2010)
Genetics and Model	Fish <i>Champocephalus gunnari</i> , fish <i>Notothenia rossii</i>	Scotia Sea, Antarctic	Genetic distance matrix; modeled migration probability matrix used to project allele frequencies after a number of generations producing a level of genetic differentiation identical to the observed level, based on a modified Bodmer and Cavalli-Sforza (1968) matrix model of migration	Not accounted	Quantitative; correlation of the observed genetic distance with predicted genetic distance	(Young et al. 2015)
Genetics, Model and Environmental variables	Kelp bass <i>Paralabrax clathratus</i> , Kellet's whelk <i>Kelletia kelletii</i> , California spiny lobster <i>Panulirus interruptus</i>	Southern California Bight	Genetic distance matrix; modeled migration probability matrix used as a metric of oceanographic distance	Not accounted	Quantitative; used linear multiple regression to predict the effects of oceanographic distance and other environmental variables on genetic distance	(Selkoe et al. 2010)
Genetics <sup>c, e, f</sup>	Coral reef fish,	Red Sea	None; study wasn't directly about	Not accounted	Qualitative	(Ben-Tzvi et

Approaches	Study taxa	Location	Dispersal metrics	Uncertainty	Type of comparison	Reference
and Elemental chemistry	<i>Neopomacentrus mirya</i> and <i>Chromis viridis</i>		dispersal but about genetic and/or chemical similarity among settling cohorts; measures were genetic and elemental similarity matrices			al. 2012)
Genetics and Elemental chemistry	Coral reef fish, <i>Amphiprion percula</i>	Papua New Guinea	None; individuals classified as self-recruiters versus dispersers based on parentage assignment	Not accounted	Qualitative; repeated measures MANOVA with individuals assigned to groups based on parentage	(Berumen et al. 2010)
Genetics and Elemental chemistry	Rainbow smelt, <i>Osmerus mordax</i>	Newfoundl and, Canada	Migration probability matrix calculated using genetic assignment; elemental signatures classified as freshwater, estuarine or marine to estimate levels of self-recruitment versus straying among tributaries	Not accounted	Qualitative	(Bradbury et al. 2008)
Genetics <sup>c</sup> and Elemental chemistry	European conger eel <i>Conger conger</i>	North-east Atlantic and Western Mediterranean	Genetic distance matrix; elemental chemistry only used for discrimination of core signatures among locations	Not accounted	Qualitative	(Correia et al. 2012)
Genetics, Elemental chemistry and Model	Yellow perch, <i>Perca flavescens</i>	Lake Erie, USA	Migration probability matrix calculated using genetic assignment; migration probability matrix calculated using and elemental assignment; hydrodynamic model used to reduce uncertainty in the origins of larvae used to characterize source population signatures	Accounted using a posterior probability threshold of 0.70 for correct assignment to source population for both genetic and elemental data	Qualitative	(Fraker et al. 2015)

Approaches	Study taxa	Location	Dispersal metrics	Uncertainty	Type of comparison	Reference
Genetics and Elemental chemistry	Neon damsel, <i>Pomacentrus coelestis</i>	Northern West Pacific	Genetic distance matrix; migration probability matrix calculated using elemental assignment	Not accounted	Qualitative	(Liu et al. 2010)
Genetics and Elemental chemistry	Black rockfish, <i>Sebastes melanops</i>	Oregon and Washington, USA	Genetic distance matrix; migration probability matrix calculated using genetic assignment; migration probability matrix calculated using elemental assignment	Not accounted	Qualitative	(Miller et al. 2005)
Genetics <sup>b</sup> and Current measurements	Limpet, <i>Cellana strigilis</i>	New Zealand, Chatham and Sub-Antarctic Islands	Genetic distance matrix; modeled dispersal probability matrix used to estimate transport times	Not accounted	Qualitative	(Chiswell 2009)
Elemental chemistry and Current measurements	Reef fish, <i>Hypsypops rubicundus</i>	California	Migration probability matrix calculated using elemental assignment; estimated dispersal distances	Not accounted	Qualitative	(Cook et al. 2014)

#### 4.8 Supplementary Information 2: Biophysical model

*Mytilus galloprovincialis* has a complex life cycle with a planktonic larval stage and sessile juvenile and adult phases. *M. galloprovincialis* can release and fertilize gametes during the whole year at intermediate latitudes, but massive spawnings are concentrated between early spring and summer, with an additional spawning peak of smaller magnitude in autumn (Villalba 1995, Caceres-Martinez and Figueras 1998, Philippart et al. 2012). The planktonic larval stage has an estimated duration ranging from 2 to 6 weeks depending mostly on temperature and food concentration (Chia et al. 1984, Lutz and Kennish 1992, Phillips 2002, Ruiz et al. 2008, Pettersen et al. 2010). Due to limited larval swimming capacity ( $\leq 0.1 \text{ cm s}^{-1}$  for bivalves (Shanks and Brink 2005)), it has been traditionally assumed that larval dispersal patterns are mostly dependent on pelagic larval duration (PLD), survival and hydrographic patterns. However, vertical migrations between layers flowing in opposite directions have been reported as a mechanism that enables larvae to regulate along- and cross-shore displacements (Metaxas 2001). Larval behaviour is a key component of larval dispersal but not completely understood (Bayne 1964). According to some authors mussel larvae concentrate at the surface during the first developmental stage (veliger) but tend to loose upward swimming velocity as development progress (pediveliger) (Fuchs and DiBacco 2011)). Light and discontinuities in the water column, like pycnoclines or food patches, are commonly suggested as relevant factors altering vertical migration behaviour (Pernet et al. 2003, Sameoto and Metaxas 2008, Daigle and Metaxas 2011) and, in general, larvae capacity for vertical position regulation might be limited to slow current speeds and low turbulence (Pernet et al. 2003, Fuchs and DiBacco 2011).

Several aspects of the species' reproductive biology were explicitly simulated by the Lagrangian component (see below) of the model, including spatial and temporal distribution of spawning intensity, larval vertical migration, larval growth rate and mortality. Four spawning scenarios (see main text) were simulated, all accounting for differences in mussel density along rocky shore habitats of the western Iberian coast (Gomes et al. 2016) but differing on spawning periodicity in order to bracket the seasonal variability (Philippart et al. 2012). Two larval behaviours were also modelled according to available information (see main text): passive behaviour (Mcquaid and Phillips 2000) and ontogenetic vertical migration (Rilov et al. 2008, Fuchs and DiBacco 2011). An unrealistic third behaviour, where larvae were forced to dwell in the bottom layer, was included in order to provide a contrast to the other two scenarios. Temperature-dependent planktonic larval duration (PLD) and mortality

were modelled pooling information from laboratory studies (Qiu et al. 2002, Sanchez-Lazo and Martinez-Pita 2012).

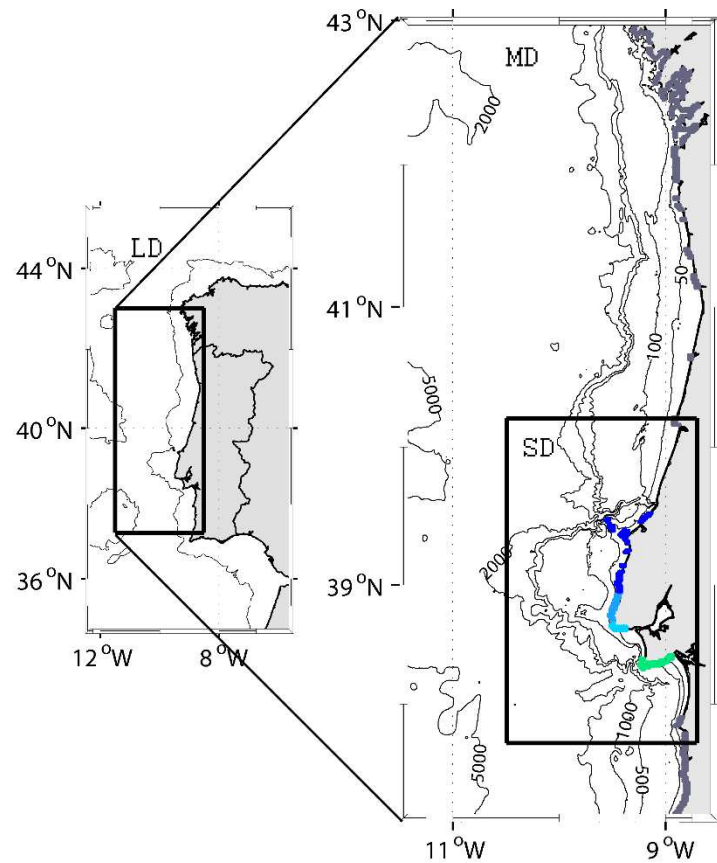
### **The oceanographic model**

The simulations were conducted using a 3-D free-surface, terrain-following primitive equation hydrostatic model configurable for fully realistic regional applications, based on the Regional Ocean Modelling System (Shchepetkin and McWilliams 2005). The present configuration represents an improvement and extension of the configuration used by Peliz et al. (2007) and Oliveira et al. (2009) to the Atlantic margin of the Iberian Peninsula, and was applied by Domingues et al. (2012) to describe (links between dispersal and supply of *Carcinus maenas* larvae to the Ria de Aveiro, and by Nolasco et al. (2013) to the study of the *Carcinus maenas* larval connectivity along the Western Iberian Margin (WIM hereinafter).

Three grids were used to resolve the circulation of the WIM (Supplementary Fig. 4.2.1): a large domain (LD), a medium domain (MD) and a small domain (SD). The LD, from 12.5° W to 5.5° W and 34.4° N to 45.5° N, has a grid resolution of 1/27° (ca. 3 km) and 60 vertical levels. This domain has been used to study the ocean circulation in the WIM by Nolasco et al. (2013), and provides initial and boundary conditions, through offline nesting, to the MD domain. The MD has a horizontal resolution of 1/60° (ca. 1,4 km) and 45 vertical levels, extending from the Cape St Vincent at 37° N to Cape Finisterre, 43° N, and from 11.5° W to the WIM coast at 8.5° W. The MD covers an area of ~670 x 245 km and constitutes the target domain used for the dispersal simulations. The SD, with a grid resolution of 1/180° (ca. 450 m) and 45 levels, was implemented in order to solve the details of the circulation in the main region where natal and recruit signatures were collected, including the Cascais and Arrábida bays. The SD domain exchange information with MD through two-way nesting, improving the circulation in the target domain MD. Tidal elevation and current ellipses, from TPXO global tide model, was applied at the boundaries of the MD (and hence SD) in order to solve the tidal dynamics in both domains. The model was run from the 1st of January until the 30th of July, 2013, with an atmospheric forcing resulting from the outputs from the Weather Research and Forecasting model (WRF, (Skamarock et al. 2008)), which was run for the same period with a 3 km resolution, and applied to LD, MD and SD domains. The inflow of freshwater to the ocean, originated from the main rivers of the region, was included in the form of realistic river outflow (provided by INAG, Water Institute of Portugal), for all the three domains. The outputs of the model, consisting of temperature, salinity, and three-

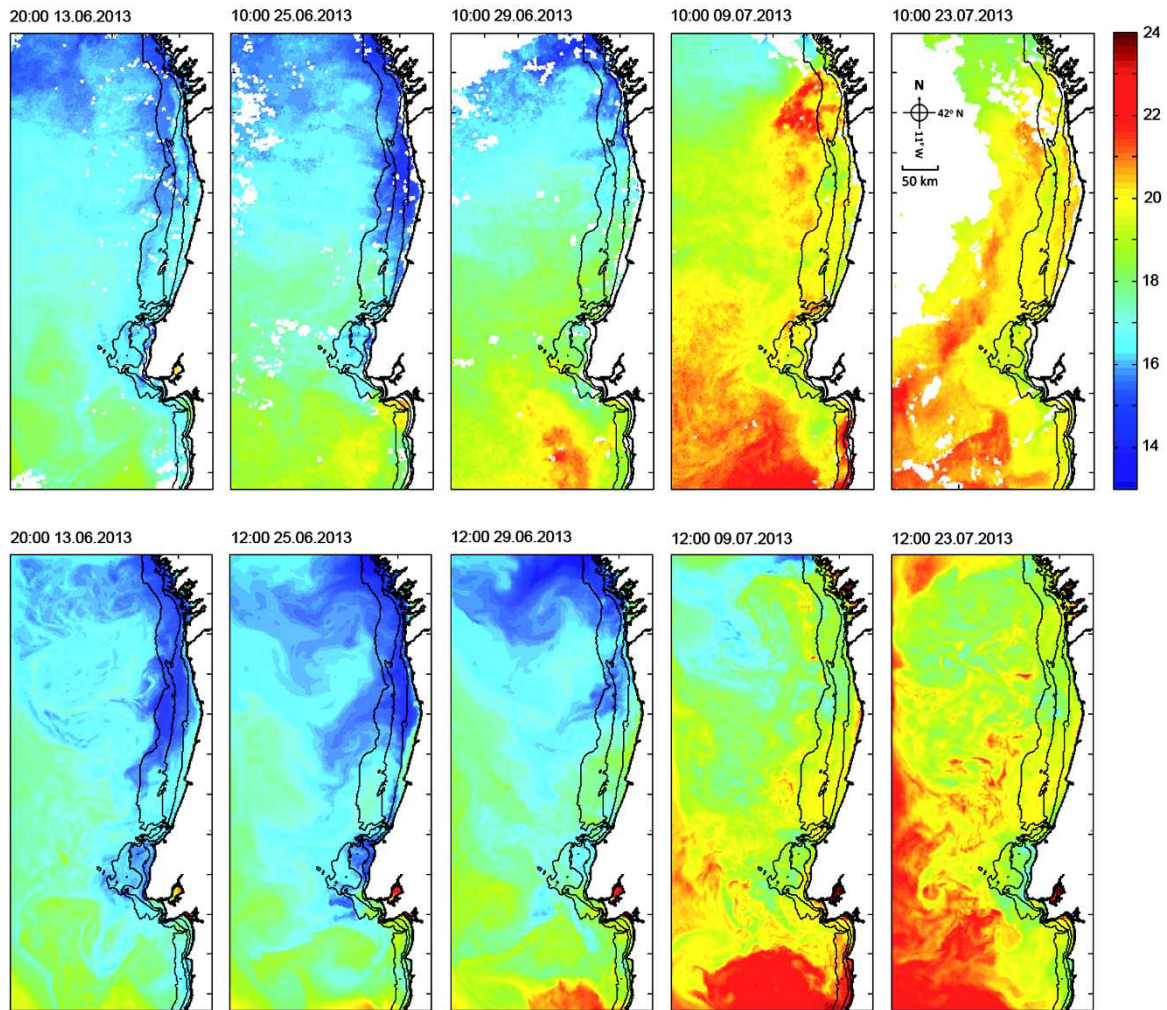
dimensional velocity fields, were stored every hour in order to be used for the Lagrangian model described below.

The performance of the LD concerning the hydrology and current fields was evaluated elsewhere (Nolasco, Pires, et al. 2013). In the present study a validation of the oceanographic model was obtained by visually comparing (Supplementary Fig. 4.2.2) sea surface temperature (SST) fields predicted for the MD, during the period covered by the study, with satellite data retrieved from the Advanced Very High Resolution Radiometer (made available by the EUMETSAT Ocean and Sea Ice Satellite Application Facility). The main features of the circulation are captured by the model, including upwelled water and filaments during the first part of the study and the poleward flow of a warmer water mass during an extended period of upwelling relaxation that started at the beginning of July.



Supplementary Figure 4.2.1 Map of the region showing the large (LD), medium (MD) and small (SD) domains. At the right panel, the MD shows the 50, 100, 500, 1000, 2000 and 5000 m bathymetric contours. The locations of rocky shore where emission/recruitment were simulated are represented in green/blue for the sampling region, and in grey for the remaining domain. This figure was produced using Python V 2.7.2 ([www.python.org](http://www.python.org)).





Supplementary Figure 4.2.2 Satellite (top row) and MD (bottom row) SST for days 13, 25 and 29 of June, and 9 and 23 of July, 2013. The colour bar (SST, °C) is the same for all the images. This figure was produced using Python V 2.7.2 ([www.python.org](http://www.python.org)).

### The biological and Lagrangian offline model

In order to simulate spawning, behaviour and growth of *M. galloprovincialis* larvae an Individual Based Model (IBM) was coupled to ROMS using ROFF (CARR et al. 2008). ROFF is a drifter-tracking code that simulates larval trajectories from stored ROMS velocity and hydrological fields. The drifter-tracking code simulates larval trajectories from stored ROMS velocity and hydrological fields using a high order predictor corrector scheme to integrate the motion equation  $dX/dt = U_{roms}(X,t)$ , with  $X$  being the position vector  $(x,y,z)$ , and  $U_{roms}$  being the modelled 3D velocity vector over time, given an initial condition  $X(t_0) = X_0$ . The time step used in this Lagrangian model,  $dt$ , is 300s. Additionally to the advection generated by the model velocities, the particle movements included random velocities in the vertical direction, which were used to parameterize unresolved turbulent processes.

Spawning and settlement were simulated along rocky coasts (Supplementary Fig. 4.2.1) using a coastal buffer strip of 2 cells for spawning and 3 cells for settlement. Spawning was made proportional to the mussel biomass at each segment of the coast (Siregar 2014) and to seasonal spawning activity (Philippart et al. 2012). This was accomplished by spawning into the model, during predicted high water, a number of virtual larvae proportional to biomass, and by varying the frequency of tides when spawning was simulated (from every high water at full spawning intensity, through 1 in every 3rd high water at low spawning intensity, to no spawning; see main text for spawning scenarios). Passive larvae were advected according to the 3D current velocities predicted by the oceanographic model. Vertically migrating larvae were forced to change layer instantaneously once the appropriate age was reached, in the case of ontogenetic behaviour, and immediately after spawning, in the case of bottom dwelling larvae, and advected at the current velocities at the respective level.

The proportional effects of temperature on PLD, and of temperature and salinity on mortality, based on the time a larva was exposed to a specific temperature in the case of PLD, or to a specific combination of temperature and salinity in the case of mortality, were estimated by linearly interpolating between the laboratory data for each larval stage. Age and the probability of death were assessed at each time step of the Lagrangian model. Larvae were killed randomly based on the proportional death rate during that time step. If a larva survived physiological stress it would grow from age 0 at spawning to age 1 at veliger and age 2 at pediveliger stages; pediveligers lived and remained competent until age 3 and then died. No other temporally or spatially distributed source of mortality (e.g. predation) was used because of lack of information.

Twelve runs of the model were performed, corresponding to four spawning scenarios and three larval behaviours. In each run, 184.340 larvae were simulated.

#### 4.9 Supplementary Information 3: matrix correlations

Supplementary Table 4.3.1 Pearson correlation coefficients between observed and modelled connectivity matrices for different combinations of larval behaviour, spawning regime, partitioning of the core region, and confidence level of the assignment of recruits into source populations. Sections A and B refer to the core matrices (without unknown row), sections C, D and E refer to the core matrices plus unknown row. A: uncorrected modelled matrix. B and C: modelled matrix corrected for Type 2 recruits. D: modelled matrix corrected for Type 3 recruits. E: modelled matrix corrected for Type 2 and Type 3 recruits. Shading indicates strength of the correlation; bold values indicate the highest correlation in each section. Pa= passive larvae. Om= larvae migrating ontogenetically. Bl= larvae dwelling in the bottom layer. S1= continuous larval emission during each high tide until July 12. S2= continuous larval emission during each high tide until June 30, then larval emission skipping one of every two high tides until July 12. S3= continuous larval emission during each high tide until June 30, then larval emission skipping two of every three high tides until July 12. S4= Continuous larval emission during each high tide until July 1, no more larvae released afterwards. 3x3, 3x4 and 4x4= spatial organization of the core region into 3 or 4 origin x destination cells. Better= recruits assigned into an origin when the probability of pertaining to that origin is better than that of pertaining to any other origin. %99, %95, %90, %75, %50= recruits assigned into an origin when the probability of pertaining to that origin is larger than the level indicated.

A)

CORE MATRICES (without unknown row); uncorrected modelled matrix		Larval behaviour / Spawning regime combinations											
		PaS1	PaS2	PaS3	PaS4	OmS1	OmS2	OmS3	OmS4	BIS1	BIS2	BIS3	BIS4
Partitioning of the core region / Confidence level combinations	3x3%99	0.64	0.72	0.77	0.87	0.58	0.67	0.75	0.82	0.28	0.31	0.34	0.38
	3x3%95	0.77	0.84	0.88	0.94	0.71	0.79	0.86	0.90	0.41	0.44	0.47	0.51
	3x3%90	0.77	0.84	0.88	0.94	0.72	0.80	0.86	0.91	0.41	0.44	0.47	0.50
	3x3%75	0.80	0.86	0.90	<b>0.96</b>	0.74	0.82	0.88	0.92	0.45	0.49	0.52	0.55
	3x3%50	0.79	0.85	0.89	0.94	0.72	0.80	0.87	0.91	0.41	0.44	0.47	0.51
	3x3 Better	0.77	0.83	0.88	0.93	0.70	0.78	0.85	0.89	0.38	0.42	0.45	0.49
	3x4%99	0.41	0.48	0.53	0.63	0.40	0.49	0.56	0.61	0.15	0.17	0.19	0.22
	3x4%95	0.61	0.67	0.71	0.79	0.59	0.67	0.73	0.78	0.33	0.36	0.38	0.41
	3x4%90	0.62	0.69	0.72	0.80	0.60	0.68	0.75	0.79	0.34	0.37	0.39	0.41
	3x4%75	0.62	0.68	0.72	0.79	0.61	0.69	0.74	0.77	0.37	0.39	0.42	0.44
	3x4%50	0.59	0.65	0.69	0.75	0.58	0.66	0.72	0.74	0.32	0.35	0.37	0.40
	3x4 Better	0.56	0.62	0.66	0.72	0.55	0.63	0.69	0.71	0.29	0.31	0.34	0.36
	4x4%99	0.40	0.47	0.50	0.60	0.38	0.46	0.52	0.56	0.13	0.15	0.17	0.19
	4x4%95	0.42	0.49	0.53	0.61	0.41	0.49	0.55	0.58	0.16	0.18	0.20	0.22
	4x4%90	0.52	0.58	0.61	0.68	0.51	0.57	0.62	0.63	0.25	0.26	0.29	0.29
	4x4%75	0.63	0.68	0.71	0.76	0.61	0.66	0.70	0.68	0.35	0.36	0.38	0.37
	4x4%50	0.66	0.71	0.73	0.78	0.64	0.69	0.73	0.70	0.38	0.38	0.41	0.40
	4x4 Better	0.65	0.70	0.72	0.76	0.64	0.69	0.72	0.69	0.37	0.38	0.40	0.38



B)

CORE MATRICES (without unknown row); modelled matrix corrected for Type 2 recruits		Larval behaviour / Spawning regime combinations											
		PaS1	PaS2	PaS3	PaS4	OmS1	OmS2	OmS3	OmS4	BIS1	BIS2	BIS3	BIS4
Partitioning of the core region / Confidence level combinations	3x3%99	0.61	0.69	0.74	0.84	0.55	0.64	0.71	0.78	0.31	0.34	0.37	0.41
	3x3%95	0.75	0.81	0.86	0.93	0.69	0.77	0.82	0.88	0.44	0.47	0.50	0.54
	3x3%90	0.75	0.82	0.86	0.93	0.69	0.78	0.83	0.88	0.44	0.47	0.50	0.54
	3x3%75	0.78	0.84	0.88	0.94	0.71	0.80	0.84	0.89	0.49	0.52	0.55	0.59
	3x3%50	0.77	0.83	0.87	0.93	0.70	0.78	0.84	0.88	0.45	0.48	0.51	0.55
	3x3 Better	0.75	0.81	0.86	0.92	0.68	0.76	0.82	0.87	0.42	0.46	0.49	0.53
	3x4%99	0.39	0.46	0.50	0.61	0.37	0.46	0.51	0.57	0.17	0.19	0.21	0.24
	3x4%95	0.59	0.65	0.69	0.78	0.56	0.64	0.70	0.75	0.36	0.38	0.40	0.43
	3x4%90	0.61	0.67	0.70	0.79	0.58	0.66	0.71	0.76	0.37	0.39	0.41	0.44
	3x4%75	0.61	0.67	0.70	0.78	0.58	0.66	0.71	0.75	0.39	0.42	0.44	0.47
	3x4%50	0.58	0.64	0.68	0.75	0.56	0.64	0.69	0.72	0.35	0.38	0.40	0.43
	3x4 Better	0.55	0.61	0.65	0.72	0.53	0.61	0.65	0.69	0.32	0.34	0.37	0.39
	4x4%99	0.48	0.54	0.57	0.65	0.48	0.55	0.61	0.62	0.17	0.19	0.21	0.23
	4x4%95	0.50	0.56	0.59	0.66	0.51	0.58	0.63	0.64	0.19	0.22	0.24	0.26
	4x4%90	0.57	0.63	0.64	0.70	0.58	0.63	0.68	0.67	0.26	0.28	0.31	0.31
	4x4%75	0.64	0.69	0.70	0.74	0.65	0.69	0.73	0.70	0.34	0.36	0.38	0.38
	4x4%50	0.66	0.71	0.71	0.75	0.67	0.71	0.74	0.70	0.37	0.38	0.41	0.40
	4x4 Better	0.65	0.69	0.70	0.73	0.66	0.70	0.73	0.69	0.35	0.37	0.39	0.39

C)

CORE MATRICES + UNKNOWNNS; modelled matrix corrected for Type 2 recruits		Larval behaviour / Spawning regime combinations											
		PaS1	PaS2	PaS3	PaS4	OmS1	OmS2	OmS3	OmS4	BIS1	BIS2	BIS3	BIS4
Partitioning of the core region / Confidence level combinations	3x3%99	0.29	0.36	0.41	0.50	0.28	0.35	0.39	0.45	0.06	0.08	0.10	0.12
	3x3%95	0.48	0.56	0.61	0.70	0.47	0.54	0.59	0.64	0.19	0.22	0.24	0.27
	3x3%90	0.61	0.68	0.74	0.82	0.59	0.67	0.72	0.77	0.26	0.29	0.31	0.34
	3x3%75	0.71	0.78	0.83	0.90	0.69	0.77	0.81	0.85	0.37	0.39	0.42	0.45
	3x3%50	0.68	0.74	0.78	0.83	0.66	0.72	0.77	0.79	0.35	0.38	0.40	0.43
	3x3 Better	0.66	0.71	0.75	0.80	0.64	0.70	0.74	0.76	0.34	0.36	0.39	0.41
	3x4%99	0.13	0.19	0.23	0.32	0.14	0.19	0.24	0.29	-0.06	-0.06	-0.04	-0.03
	3x4%95	0.32	0.39	0.44	0.54	0.31	0.38	0.44	0.49	0.07	0.09	0.10	0.12
	3x4%90	0.44	0.52	0.57	0.68	0.42	0.50	0.58	0.64	0.16	0.18	0.19	0.21
	3x4%75	0.58	0.65	0.70	0.80	0.55	0.63	0.70	0.76	0.28	0.30	0.32	0.33
	3x4%50	0.57	0.64	0.67	0.74	0.54	0.61	0.67	0.71	0.30	0.32	0.34	0.35
	3x4 Better	0.55	0.60	0.64	0.71	0.52	0.58	0.64	0.68	0.28	0.30	0.32	0.33
	4x4%99	0.59	0.60	0.60	0.61	0.55	0.56	0.57	0.56	0.46	0.45	0.47	0.42
	4x4%95	0.64	0.67	0.67	0.70	0.60	0.63	0.65	0.66	0.47	0.46	0.48	0.44
	4x4%90	0.68	0.72	0.73	0.77	0.65	0.68	0.72	0.73	0.46	0.46	0.48	0.44
	4x4%75	0.72	0.77	0.79	0.84	0.69	0.73	0.78	0.80	0.44	0.44	0.47	0.43
	4x4%50	0.54	0.60	0.64	0.72	0.52	0.58	0.63	0.68	0.22	0.24	0.26	0.25
	4x4 Better	0.66	0.71	0.75	0.80	0.64	0.70	0.74	0.76	0.34	0.36	0.39	0.41

D)

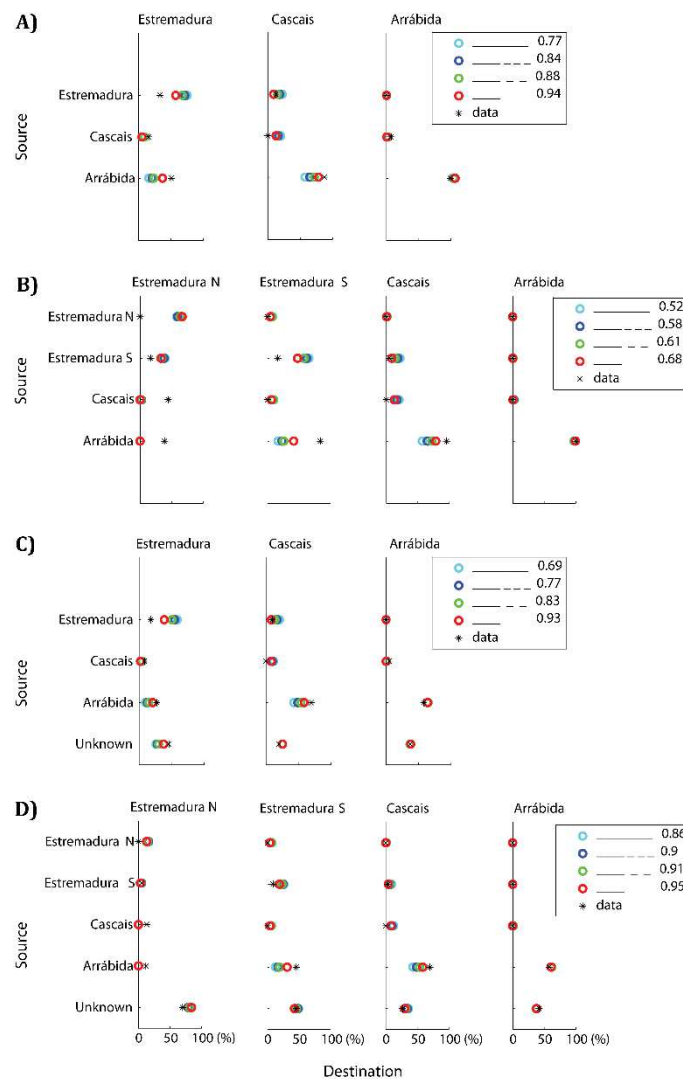
CORE MATRICES + UNKNOWNNS; modelled matrix corrected for Type 3 recruits		Larval behaviour / Spawning regime combinations											
		PaS1	PaS2	PaS3	PaS4	OmS1	OmS2	OmS3	OmS4	BIS1	BIS2	BIS3	BIS4
Partitioning of the core region / Confidence level combinations	3x3%99	0.21	0.27	0.34	0.47	0.22	0.30	0.35	0.43	-0.13	-0.11	-0.09	-0.06
	3x3%95	0.43	0.50	0.56	0.68	0.43	0.50	0.56	0.62	0.05	0.07	0.09	0.13
	3x3%90	0.61	0.68	0.75	0.84	0.60	0.68	0.74	0.79	0.20	0.22	0.25	0.29
	3x3%75	0.79	0.85	0.91	0.97	0.76	0.83	0.88	0.90	0.42	0.44	0.48	0.51
	3x3%50	0.82	0.87	0.90	0.91	0.77	0.82	0.86	0.85	0.51	0.53	0.56	0.59
	3x3 Better	0.79	0.84	0.87	0.88	0.74	0.79	0.83	0.81	0.50	0.52	0.55	0.58
	3x4%99	0.31	0.35	0.40	0.49	0.28	0.33	0.38	0.43	-0.22	-0.21	-0.20	-0.18
	3x4%95	0.48	0.54	0.59	0.67	0.44	0.50	0.56	0.60	-0.04	-0.03	-0.01	0.02
	3x4%90	0.59	0.65	0.70	0.78	0.53	0.61	0.67	0.71	0.11	0.13	0.15	0.18
	3x4%75	0.67	0.74	0.77	0.84	0.62	0.70	0.76	0.78	0.33	0.35	0.37	0.40
	3x4%50	0.56	0.62	0.64	0.67	0.52	0.59	0.64	0.65	0.44	0.45	0.47	0.49
	3x4 Better	0.50	0.56	0.58	0.61	0.47	0.54	0.58	0.59	0.43	0.44	0.46	0.48
	4x4%99	0.42	0.46	0.50	0.56	0.38	0.42	0.46	0.50	-0.15	-0.13	-0.12	-0.10
	4x4%95	0.54	0.58	0.63	0.70	0.50	0.55	0.60	0.65	-0.05	-0.03	-0.02	0.00
	4x4%90	0.61	0.66	0.70	0.77	0.56	0.62	0.67	0.72	0.04	0.06	0.08	0.09
	4x4%75	0.69	0.74	0.78	0.84	0.64	0.71	0.76	0.79	0.23	0.24	0.26	0.26
	4x4%50	0.60	0.66	0.68	0.72	0.56	0.62	0.67	0.68	0.42	0.42	0.45	0.43
	4x4 Better	0.57	0.62	0.63	0.66	0.54	0.59	0.63	0.64	0.44	0.44	0.47	0.45

E)

CORE MATRICES + UNKNOWNNS modelled matrix corrected for Type 2 and Type 3 recruits		Larval behaviour / Spawning regime combinations											
		PaS1	PaS2	PaS3	PaS4	OmS1	OmS2	OmS3	OmS4	BIS1	BIS2	BIS3	BIS4
Partitioning of the core region / Confidence level combinations	3x3%99	0.47	0.55	0.61	0.76	0.51	0.58	0.62	0.71	0.07	0.09	0.11	0.14
	3x3%95	0.63	0.71	0.77	0.88	0.65	0.72	0.76	0.83	0.20	0.23	0.25	0.28
	3x3%90	0.69	0.77	0.83	0.93	0.71	0.78	0.83	0.88	0.26	0.29	0.32	0.35
	3x3%75	0.70	0.77	0.82	0.88	0.70	0.77	0.82	0.83	0.36	0.39	0.42	0.45
	3x3%50	0.57	0.63	0.66	0.66	0.55	0.61	0.65	0.63	0.35	0.37	0.39	0.41
	3x3 Better	0.53	0.58	0.62	0.61	0.50	0.56	0.60	0.57	0.33	0.35	0.38	0.40
	3x4%99	0.55	0.60	0.63	0.73	0.52	0.57	0.61	0.67	-0.06	-0.04	-0.03	-0.02
	3x4%95	0.63	0.70	0.74	0.83	0.60	0.66	0.71	0.76	0.08	0.10	0.11	0.13
	3x4%90	0.63	0.70	0.74	0.83	0.59	0.66	0.72	0.78	0.16	0.18	0.20	0.22
	3x4%75	0.56	0.63	0.67	0.73	0.54	0.61	0.67	0.70	0.28	0.30	0.31	0.33
	3x4%50	0.32	0.37	0.39	0.42	0.31	0.36	0.41	0.42	0.30	0.31	0.33	0.34
	3x4 Better	0.25	0.30	0.32	0.35	0.24	0.30	0.34	0.35	0.28	0.29	0.31	0.32
	4x4%99	0.89	0.90	0.90	0.92	0.84	0.85	0.86	0.86	0.47	0.46	0.48	0.43
	4x4%95	0.89	0.92	0.93	0.96	0.86	0.88	0.90	0.92	0.48	0.47	0.49	0.45
	4x4%90	0.86	0.90	0.91	0.95	0.83	0.86	0.89	0.91	0.47	0.46	0.49	0.45
	4x4%75	0.75	0.79	0.82	0.86	0.73	0.78	0.81	0.83	0.45	0.44	0.47	0.43
	4x4%50	0.33	0.38	0.41	0.46	0.32	0.38	0.43	0.45	0.22	0.23	0.25	0.25
	4x4 Better	0.21	0.26	0.29	0.34	0.21	0.27	0.32	0.34	0.14	0.15	0.17	0.17

#### 4.10 Supplementary Information 4: matrix adjustment

Accounting for uncertainty increases the fit between observed and modelled connectivity matrices at higher spatial resolutions. In Supplementary Fig. 4.4.1 A) and B), the 3x3 spatial grid yields the best adjustment between the observed and modelled matrices. In Supplementary Fig. 4.4.1 C) and D), the 4x4 spatial grid yields the best correlations between the observed and modelled matrices. Accuracy greatly increases in the Estremadura regions for the corrected case.

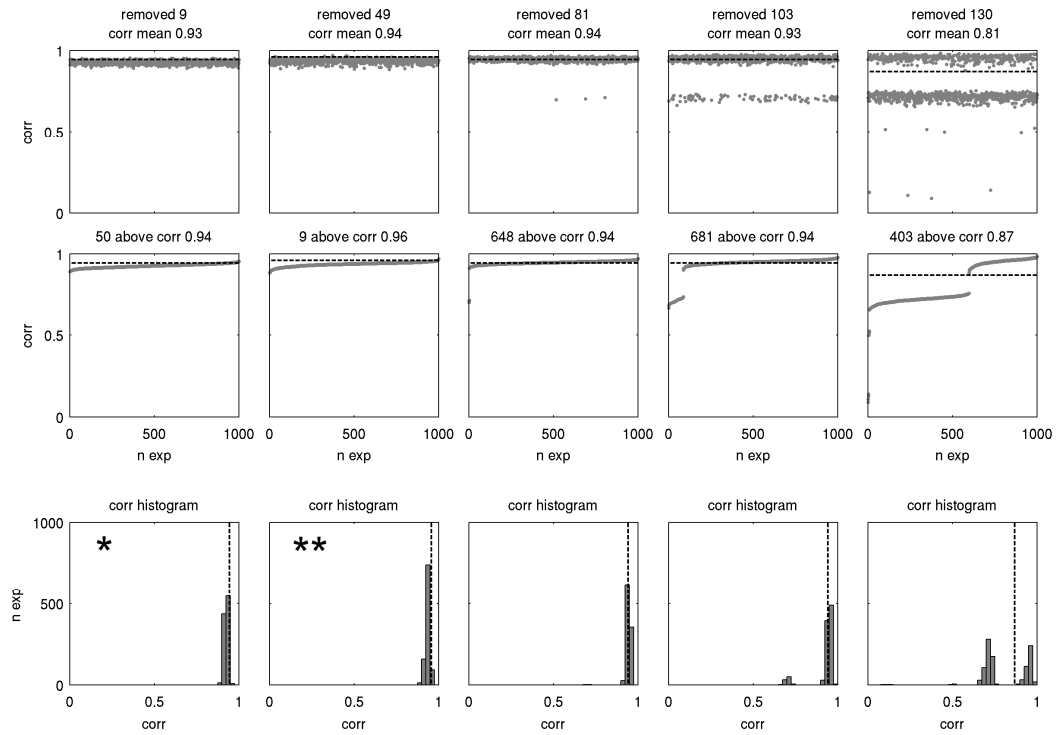


Supplementary Figure 4.4.1 Graphical comparison between Observed and Modelled connectivity matrices with two different spatial grid arrangements, for passive behaviour, 90% confidence level and four spawning regimes (colours). Data indicates observations. A) 3x3 core connectivity matrices uncorrected for Type 2 and/or Type 3 recruits; B) 4x4 core connectivity matrices uncorrected for Type 2 and/or Type 3 recruits; C) 3x3 core connectivity matrices corrected for Type 2 and Type 3 recruits plus; D) 4x4 core connectivity matrices corrected for Type 2 and Type 3 recruits plus.

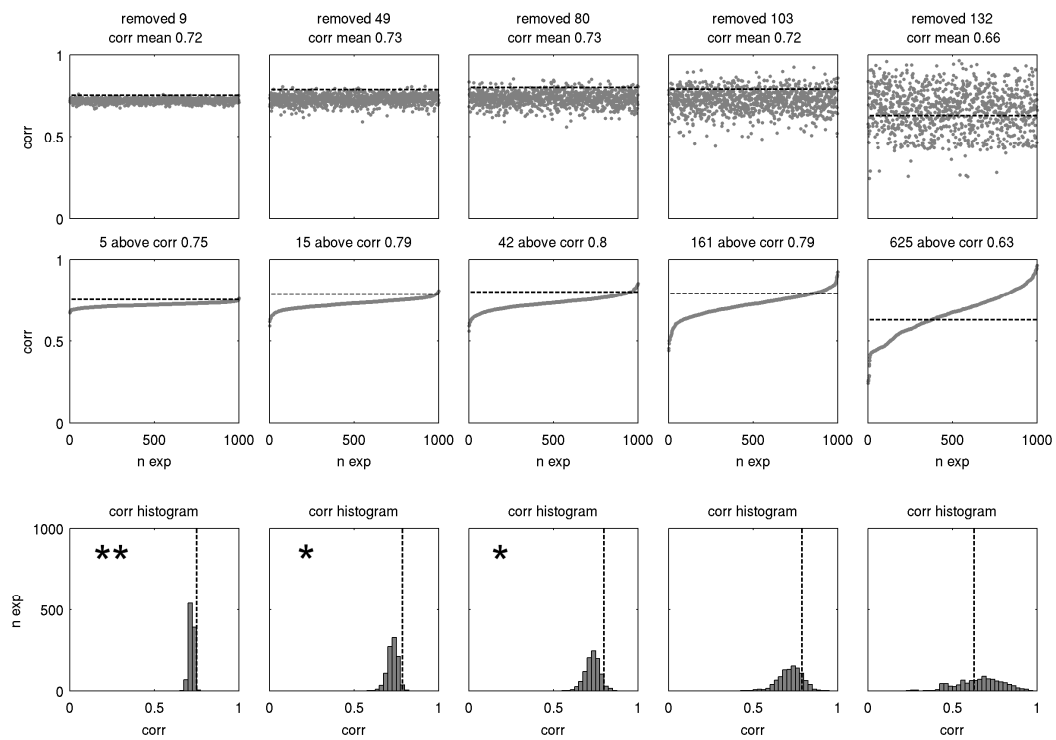
unknown row; D) 4x4 core connectivity matrices corrected for Type 2 and Type 3 recruits plus unknown row.

#### 4.11 Supplementary Information 5: prediction

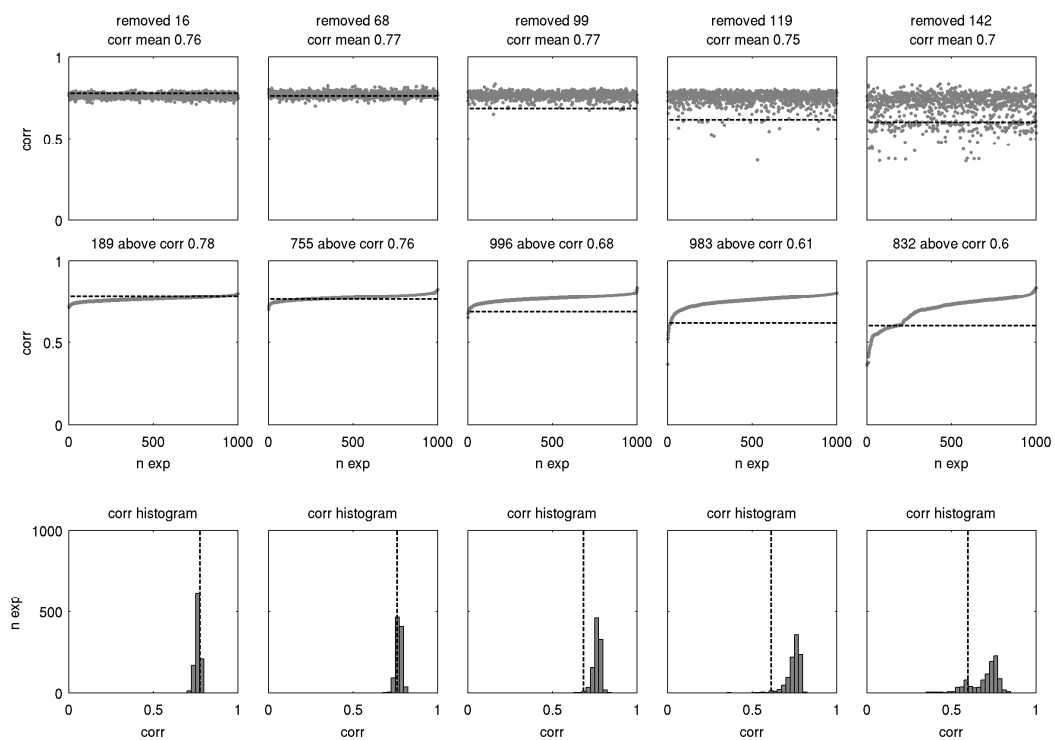
A)



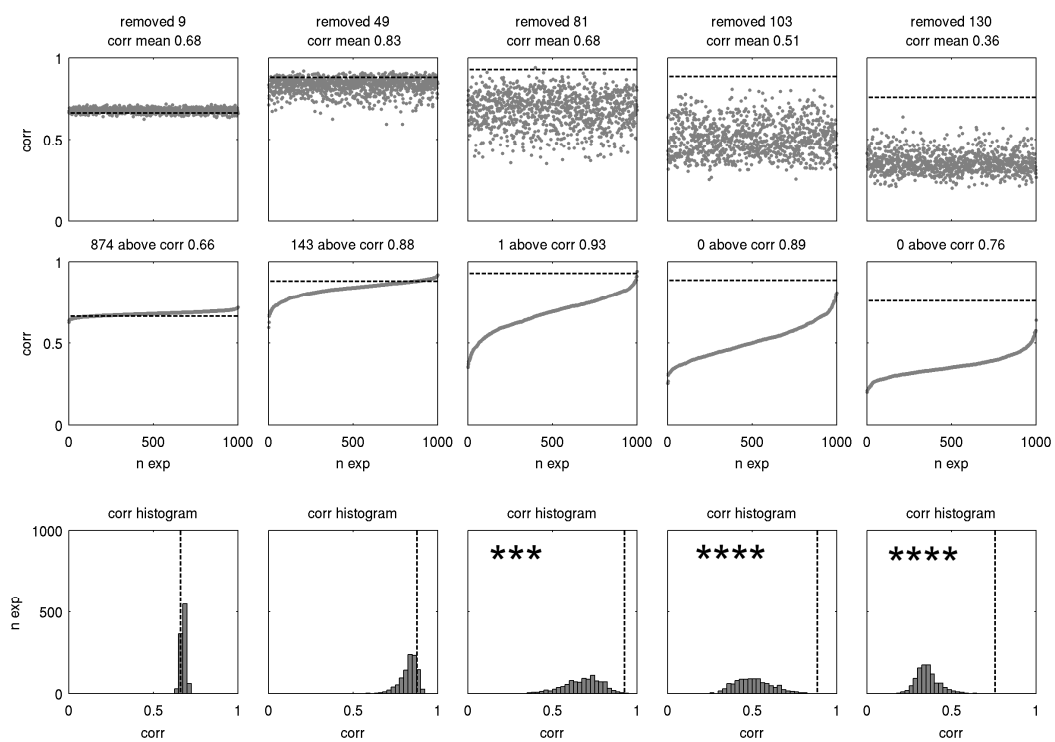
B)



c)

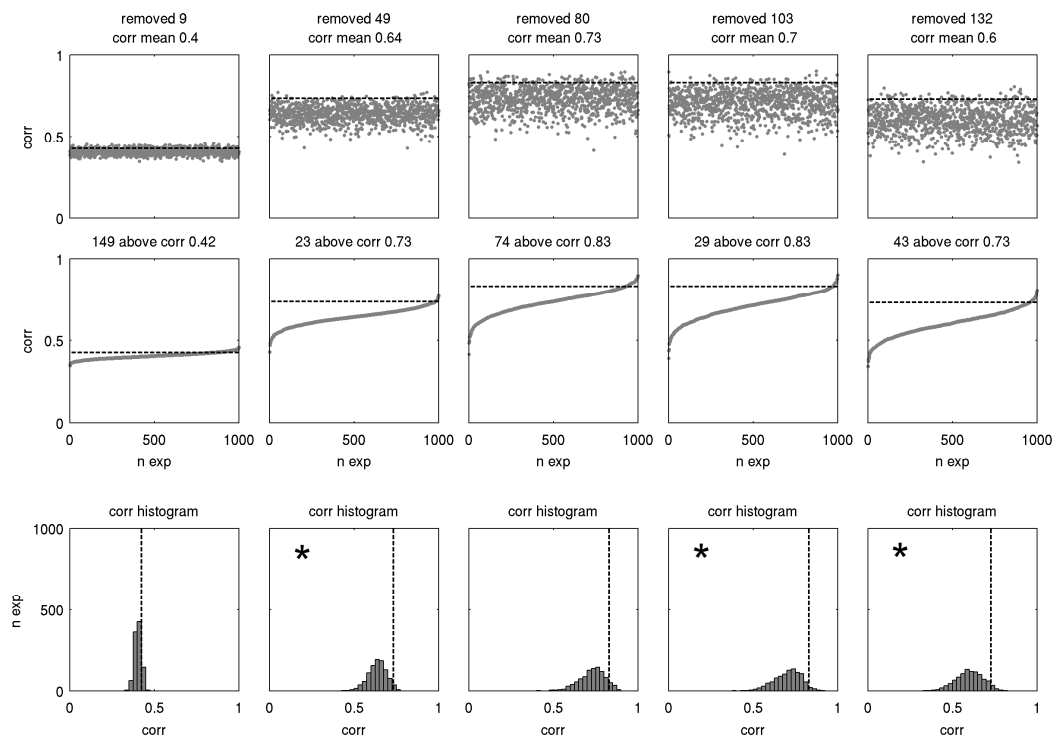


D)

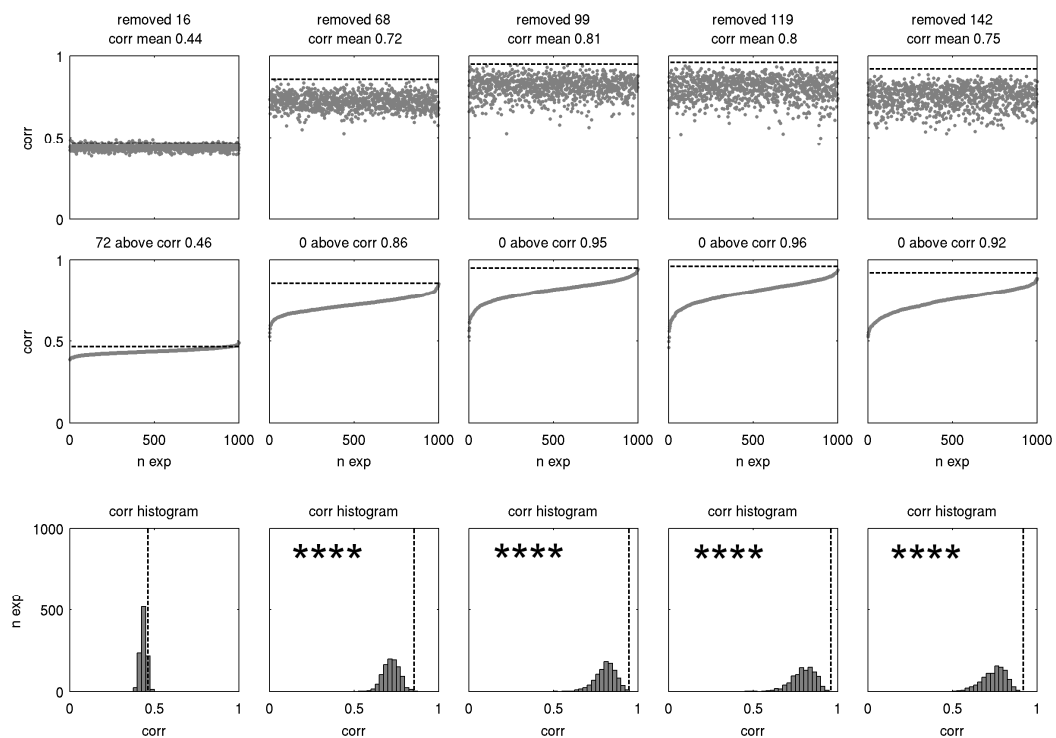




E)



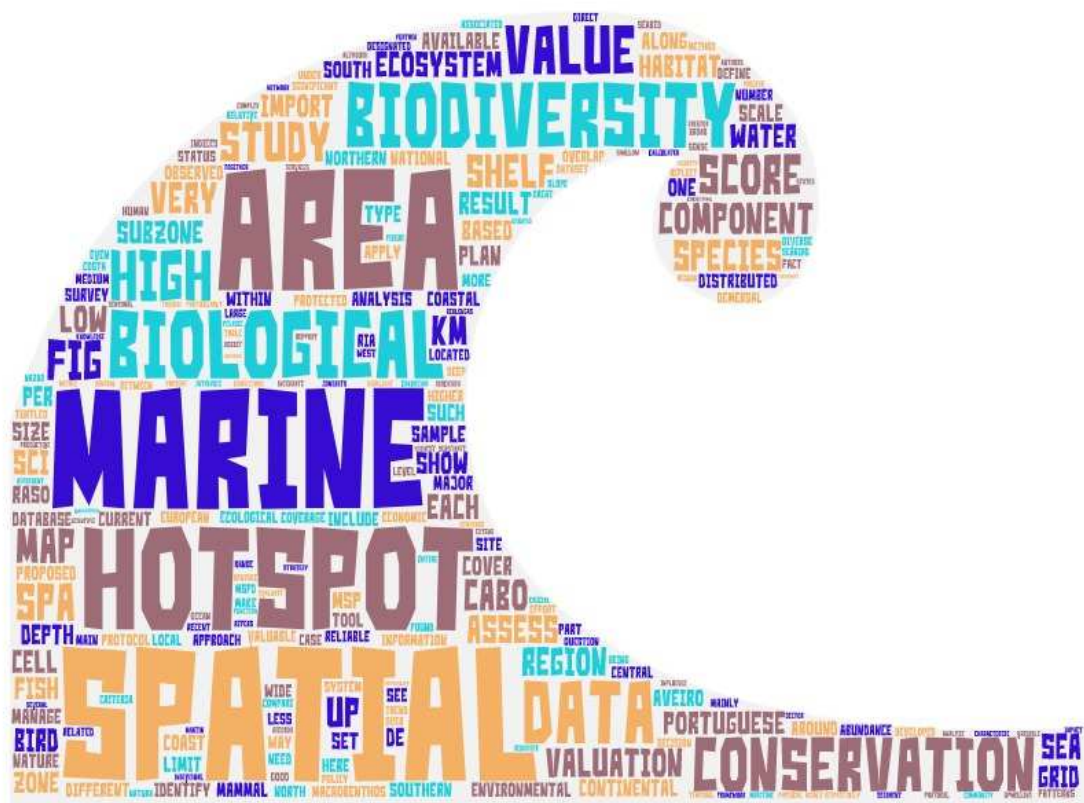
F)



Supplementary Figure 4.5.1. Effect on the matrix correlation coefficient of randomly excluding from the observed matrix a number of recruits equal to the number of observed recruits correctly classified as unknowns for each confidence level (columns are Assignment Probability Thresholds (APT) of 0.50, 0.75, 0.90, 0.95 and 0.99), based on 1000 trials for each threshold. Only the cases for continuous larval emission during each high tide until July 1 (S4) and passive larvae (Pa) were tested. A) 3x3 spatial arrangement, core connectivity matrices; B) 3x4 spatial arrangement, core connectivity matrices; C) 4x4

spatial arrangement, core connectivity matrices; D) 3x3 spatial arrangement, connectivity matrices with unknown row; E) 3x4 spatial arrangement, connectivity matrices with unknown row; F) 4x4 spatial arrangement, connectivity matrices with unknown row. In panels D) - F) the unknown row is composed of the recruits that were randomly assigned as unknowns in the case of the observed matrix, and of Type 2 and Type 3 recruits in the case of the modelled matrix.

In each graph, the dashed line indicates the correlation coefficient that was obtained by removing those recruits that correctly failed to pass the posterior probability threshold. First row of each panel: distribution of correlation coefficients ranked by trial number; the number of removed individuals is indicated above each graph. Second row of each panel: the same, but correlation coefficients ranked by value; the number of trials with a correlation coefficient above that obtained by removing those recruits that correctly failed to pass the posterior probability threshold is indicated above each graph. Third row of each panel: frequency distribution of the correlation coefficients. Removing the recruits that correctly failed to pass the APT resulted in a correlation coefficient significant higher than that obtained by a random deletion of recruits, at  $p < 0.05$ , 0.01, 0.001 and 0.0001 (\*, \*\*, \*\*\*, \*\*\*\*, respectively). "corr"= correlation coefficient.



## Chapter V

## Marine biological value along the Portuguese continental shelf; insights into current conservation and management tools

Inês Gomes I, Pérez-Jorge S, Peteiro L, Andrade, Bueno-Pardo J, Quintino V, Rodrigues AM, Azevedo M, Vanreusel A, Queiroga H, Deneudt K. 2018 Marine biological value along the Portuguese continental shelf; insights into current conservation and management tools. *Ecological Indicators* (under review)

**Abstract**

The valuation of nature is an inbuilt component of validating environmental management decisions and an important research field for different disciplines related to conservation, economy and ethics. Here, biodiversity was valued using an ecological approach based on the intrinsic value incorporated in biodiversity *per se*, regardless of any human association. The Marine Biological Valuation protocol was drawn upon the methodology of terrestrial valuation maps, to support the European MSFD environmental status assessment (descriptor 1 – biodiversity) and national marine spatial planning approaches. To apply the protocol on the Portuguese continental shelf we compiled and analyzed national biological databases for a wide taxonomic range of ecosystem components (seabirds, demersal fish, macrobenthos, marine mammals and sea turtles) and assessed the spatial overlap with existing and proposed conservation areas (Natura 2000 network). The resultant maps described patterns of biological value consistent with the physical and biological oceanographic conditions as well as local hydrodynamics of the Portuguese continental shelf. The results of our approach confirm previously identified valuable areas for protection (particularly in the northern and central regions), but also highlights the value of currently unprotected sites, mainly in the southern region. Biological valuation maps showed to be comprehensive tool to compile and spatially analyze biological datasets. By drawing attention to subzones of biological importance, it constitutes a valuable instrument in making appropriate-scale decisions on the spatial allocation of human activities in the context of the Portuguese marine spatial planning, currently facing the pressure and impacts of increased maritime exploitation.

**Keywords:** assessment tool, intrinsic value, biodiversity, ecological criteria, conservation, marine spatial planning

## 5.1 Introduction

Biological diversity is recognised as the foundation of healthy and multifunctional ecosystems (Hector and Bagchi 2007, Worm et al. 2006) and its conservation an important aim of environmental management (Brooks et al. 2006, Selig et al. 2014). The valuation (or “attributing importance/weight”) of nature is an inbuilt component of validating environmental management decisions. Although the quantification of the wide-ranging value of biodiversity is currently a significant subject of investigation for conservation, economy and ethics disciplines, the tools, methodologies and outcomes have yet to reach a consensus amongst researchers.

In fact, much debate still surrounds the concepts of biological diversity and biodiversity itself. The challenge is then to find ways to evaluate the multidimensional diversity concepts (including all biotic variation from genes to ecosystems level) in useful and operational ways (Purvis and Hector 2000).

In its broad sense, biodiversity is valued regarding the views of anthropocentrism or ecocentrism, as having a transaction and/or utility value (economic and/or social relation to humans) or holding an intrinsic biological value. Valuing nature requires therefore a complex combination of distinct value perspectives; economic, socio-cultural and ecological (Laurila-Pant et al. 2015, Scholte et al. 2015). There is an ongoing debate about the methods to perceive and value nature to reflect a realistic and integrative contribution of biodiversity in decision making (Chan et al. 2016, Jacobs et al. 2016).

Valuing biodiversity and ecosystem services in monetary terms (assigning a metric value to ecosystem components and functions which benefit humankind (Costanza et al. 1997) is a contemporary trend (Kubiszewski et al. 2017) enshrined into a number of international frameworks, such as the European Union 2020 Biodiversity Strategy, the Intergovernmental Platform on Biodiversity and Ecosystem Services (IPBES), the Millennium Ecosystem Assessment (MA) and in marine policies like the European Marine Strategy Framework Directive (MSFD). Even though there are several definitions and classification systems to economically value biodiversity in the literature (see de Groot et al. 2002), no unified framework has been reached to assess, measure, and integrate marine monetary metrics in environmental management (Nahlik et al. 2012). Monetary evidences are believed to be easily conveyed to a broad audience and assimilated into conservation policy-making processes (Bräuer 2003). Also, economic valuation of nature can be a pragmatic way forward to add to scientific and ethic approaches to reach conservation goals; a strategy used in other domains like public health, development and education (Scharks and Masuda 2016). Several studies

have already economically calculated coastal and marine ecosystem services in different settings: in estuarine waters (Barbier et al. 2011), coral reefs (Pendleton 1995), artificial reefs (Polak and Shashar 2013), mangrove forests (Huxham et al. 2015), sea grass meadows (Tuya et al. 2014), open sea (Ressurreição et al. 2011) and the deep sea (Jobstvogt et al. 2014). However, most critics to environmental economic valuation point out the fact that many financial proxies cannot reflect the highly complex and dynamic role of biodiversity and ecosystem services to human wellbeing (Bartkowski et al. 2015). This is especially true in the marine setting, with physical and biological fundamental differences when compared to the terrestrial environment (Carr et al. 2003). For instance, the relative "openness" of marine populations (i.e. greater magnitudes and higher rates of import and export than their terrestrial counterparts) along with the way anthropogenic pressures are more diffuse in the highly dynamic tri-dimensional matrix (from the surface to the subsoil), require broader spatial and temporal scale approaches to value biodiversity in ecologically meaningful ways. Also, several arguments have emerged among conservationists that conventional economic approaches are inadequate for conservation issues since they quantify ecosystem services as marketable, and consequently, replaceable commodities (Gómez-Baggethun et al. 2010, Peterson et al. 2010) contradicting conservation targets (Callicott et al. 2015). Spash (2015) argued that this economic logic of natural systems and its offset principle, does not seek to prevent or reduce biodiversity devastation, but to legitimize it.

A complementary approach values biodiversity through its non-use, intangible socio-cultural value. It investigates personal attitudes and perceptions regarding ecosystem services in non-monetary terms (Daniel et al. 2012, Kenter et al. 2015). These valuation techniques are however constrained to landscapes greatly shaped by human direct influence (Martin-López et al. 2012) and less competent in offshore marine areas (but see Christie et al. 2017). In the marine environment, the quantification of this socio-cultural component has been mainly treated within the context of marine protected areas (Angulo-Valdés and Hatcher 2010, Petrosillo et al. 2007).

Finally, the ecological approach to value of biodiversity is based on the intrinsic value of biodiversity *per se*, regardless of any human association. This notion has been the basis not only for environmental ethics but also for biological conservation disciplines. Whether it is based on a philosophical view, or supported by available scientific methods or judgment, intrinsic values in nature are now widely accepted by conservationists (Cafaro and Primack 2014, Doak et al. 2014, Vucetich et al. 2015). In order to reduce the subjectivity of "inherent values", various systematic decision supporting tools have been developed, using multiple biodiversity metrics and spatial analysis to meet the conservation targets (e.g. Aíramé et al.

2003, Villa et al. 2002). Some studies identify areas of ecological importance, focusing on individual taxa (Fishpool et al. 1998), groups of species (Eken et al. 2004), or habitats (Ward et al. 1999), using multiple ecological criteria (Roberts et al. 2003) or highlighting hotspots of rare/endemic species or high species richness (Myers et al. 2000). At a global scale, the Convention on Biological Diversity (CBD) has adopted a scheme to recognize 'Ecologically or Biologically Significant Marine Areas' (EBSAs) in need of protection. Seven scientific criteria are used to define EBSAs (Dunn et al. 2014): uniqueness or rarity; special importance for life-history stages; importance for threatened, endangered or declining species and/or habitats; vulnerability, fragility, sensitivity, or slow recovery; biological productivity; biological diversity; and naturalness.

The Marine Biological Valuation protocol presented here (Deraus et al. 2007a, 2007b) was drawn upon the methodology of the terrestrial valuation maps, to fulfill the emergent need on solid spatial information to support marine spatial planning approaches. The protocol developed by Derous (2007c) uses valuation criteria based on a thorough review of academic literature and international legislative documents on marine biological assessment by a panel of experts from Project BWZee - A Biological Valuation Map for the Belgian Continental Shelf. Unlike the EBSA protocol, whose aim was to identify areas in need of protection, including criteria related to human impacts, the method reflects on "the inherent value of marine biodiversity, without reference to anthropogenic use". It was initially developed for the Belgian part of the North Sea, but has also been applied to the shallow Belgian coastal zone (Vanden Eede et al. 2014), UK (Vanden Eede 2007), Azores (Rego 2007), Denmark (Forero 2007) and Spain (Pascual et al. 2011). Also, Weslawski et al (2009) used a modified version of this protocol to assess the biological value of the benthic communities in the southern Baltic Sea.

This protocol was applied in this paper to generate marine biological valuation maps for the continental Portuguese shelf, using available biological datasets. These maps can serve as integrative baseline information within the European MSFD environmental status assessment (descriptor 1 – biodiversity) and to define priority conservation areas in marine spatial planning (MSP).

Meaningful Initiatives which integrate existing full spatial coverage biological datasets are crucial for the monitoring of biodiversity, given the contemporary pressure on marine resource exploitation and the competitiveness of the maritime economy (Golden et al. 2017). This is particularly true in the Portuguese case, having one of the largest continental shelf areas in the European Union and where the National Ocean Strategy 2013-2020 is set on the "blue growth" development model, promoting greater efficiency in the use of marine resources. The Portuguese MSP plan establishes the legal basis for the national policy on marine spatial

planning and management, using the “Plano de Ordenamento do Espaço Marítimo POEM 2008-2012” (INAG, 2012) as the national reference situation for coastal and ocean planning and private use. However, concerns have arisen that the framework is mainly driven by economical concerns, with environmental conservation coming second to economic goals (Frazão Santos et al. 2015, 2014). Calado et al. (2010) stated that the major operational challenge encountered in developing the Portuguese MSP was the access to suitable quality data (and associated metadata) and the lack of implementation tools to facilitate an effective public discussion. In this sense, the specific objectives of this work are: (i) to explore, compile and summarize national marine biological databases: (ii) to apply the marine biological valuation approach on the Portuguese continental shelf waters (iii) to assess the spatial overlap of the valuation scores with marine conservation areas (Natura 2000 network) and (iv) to examine the significance of our results in the context of the Portuguese marine spatial planning. To our knowledge this is the first published attempt to combine and spatially evaluate data for a wide taxonomic range of ecosystem components (seabirds, demersal fish, macrobenthos, marine mammals and sea turtles) at the scale of tens of kilometers along the continental Portuguese shelf.

## **5.2 Material and Methods**

### **5.2.1 Study area**

In this study we used existing georeferenced databases for the distribution and abundance of marine organisms in the continental Portuguese shelf waters. The Portuguese continental shelf extends from the Galicia Bank to the Gulf of Cadiz for approximately 900 km in length, averaging a width of approximately 45 km, and is bordered by an irregular and steep shelf-break at around 160 m (Figure 5.1). The shelf is characterized by a variety of sediment types (Martins. et al. 2012) and cleaved by three main deep submarine canyons Nazaré, Cascais/Lisbon and Setúbal, representing geo-morphological and hydrological margins (Oliveira et al. 2007). In the western margin, the shelf northern sector is moderately wide (up to 60 km), and receives significant input from rivers, being a high-energy environment exposed to NW swells and high biological productivity. Distinctively, the southern sector (about 10 to 20 km wide), receives less riverborne input, has a steeper slope and is subjected to a low energy regime with swells predominantly from SW-S and SE (Mil-homens et al. 2007). In the southern margin the continental shelf is generally narrow and further characterized by relatively shallow depths (110 to 150m) of the shelf break. Being situated at the northern limit of the Eastern North Atlantic Upwelling Region, the Portuguese continental coast is also strongly influenced



by seasonal upwelling events (Relvas et al. 2007): from approximately June to October, the upwelling system brings cold and nutrient-rich waters to the surface, while warmer offshore waters reach the shelf from November to May.

Our study area covered 41866.5 km<sup>2</sup>, representing 13% of the Portuguese economic exclusive zone (EEZ, 327 667 km<sup>2</sup>). Since it covers a large area with great topographic and oceanographic variability, it is subdivided for this study into 4 main regions (northern, central, southwestern and southern) to assist in describing and interpreting the results). For further analysis, each region was divided into grid cells of 9 km x 9 km (see Figure 5.1). These grid cells were defined as subzones within the study area which could be scored relative to each other, against a set of biological valuation criteria. At first, the subdivision of the study area according to a habitat classification was considered, but the highly heterogeneous marine benthic substratum type (Martins et al. 2012) hindered to have representative habitat types as subzones, at the scale of this study. The applied grid and the size of the subzones (grid cells size) was then chosen taking into consideration the total size of the study area, the sampling effort of the available data and on the basis of ecologically-meaningful parameters, like the mobility and dynamics of the biodiversity component under consideration. Even though smaller grid cells would make more sense in the case of relatively immobile benthic organisms when compared to highly mobile birds or marine mammals, the considerably lower sampling effort subjacent to some datasets led us to the decision of using an equally sized grid cell for all components.

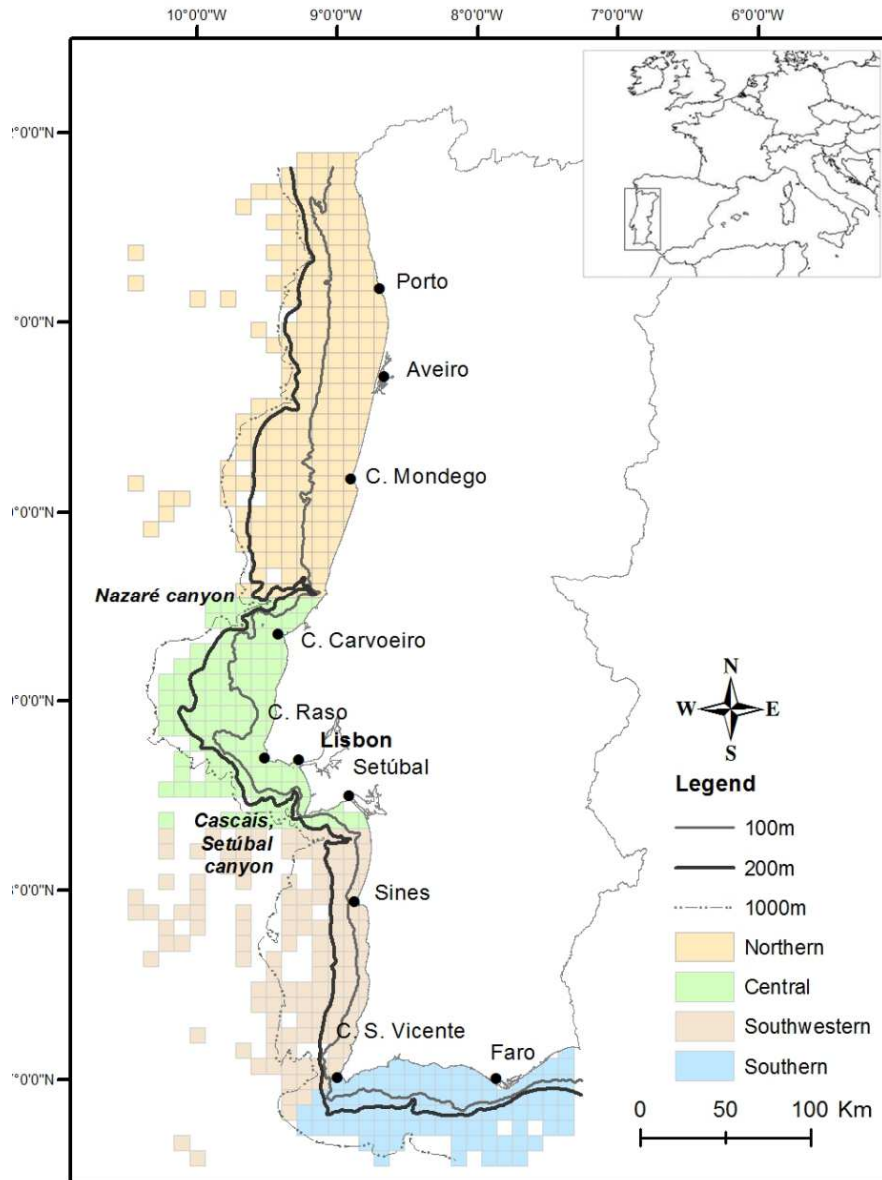


Figure 5.1 Overview of the study area illustrating the subzones used for biological valuation (grid cells 9 km x 9 km) around the Portuguese continental shelf waters. The colour scheme represents the region limits used to assist in interpreting the results. Bathymetric lines show the 100 m (dark grey), 200 m (black) and 1000 m (dashed line) depth contours. Some important topographic features and locations cited in the text are also shown.

### 5.2.2 Databases

This study included five marine ecosystem components (macrobenthos, birds, demersal fish, marine mammals and sea turtles) for which sufficient and adequate spatial distribution data were available for the Portuguese continental shelf (Table 5.1).

Table 5.1 Sampling period, method, number of records, number of species and selected and ecologically significant and habitat forming species per ecosystem component. Number of subzones with data and percentage (out of the total number of subzones). Spearman correlation between the biological valuation scores per ecosystem component and the total biological valuation score.

Ecosystem component	Sampling period	Sampling method	Nr of records	Nr of species	Ecologically significant species	Habitat forming species	Number of subzones with data (% of total)	Spear. corr.
Macrobentos	2007 (Martins et al 2013)	145 stations (0.1 m <sup>2</sup> Smith–McIntyre grab, sieved on board over 1 mm mesh size)	6526	603	<i>Protodorvillea kefersteini</i> , <i>Pisone remota</i> , <i>Asbjornsenia pygmaea</i> , <i>Magelona johnstoni</i> , <i>Urothoe pulchella</i> , <i>Fabulina fabula</i> , <i>Abra alba</i> , <i>Galathowenia oculata</i> , <i>Lumbrinerides amourexii</i> , <i>Euchone rubrocincta</i> , <i>Lysidice unicornis</i> , <i>Sternaspis scutata</i> , <i>Heteromastus filiformis</i> , <i>Psammogammarus caecus</i>	<i>Lanice conchilega</i> , <i>Sabellaria spinulosa</i>	115 (21%)	0.46
Birds	2004-2012 SPEA	15818 observations (European Seabirds at Sea -ESAS- protocols)	15819	67	<i>Puffinus mauretanicus</i> , <i>Calonectris diomedea</i> , <i>Morus bassanus</i> , <i>Larus michahellis</i>		534 (97%)	0.80
Demersal Fish	2008 IPMA (Chaves et al 2008)	88 stations (Bottom trawl surveys, average of 3.5 knots, each haul lasting 30 minutes. Mesh size of 20 mm.	1494	156	<i>Abralia (Asteroteuthis) veranyi</i> , <i>Alloteuthis, Illex coindetii</i> , <i>Loligo vulgaris</i> , <i>Sepiolidae</i> , <i>Todaropsis eblanae</i> , <i>Engraulis encrasicolus</i> , <i>Sardina pilchardus</i>		86 (16%)	0.25
Marine Mammals and Sea Turtles	2004-2012 SPEA	581 observations (ESAS protocol)	582	16	<i>Balaenoptera acutorostrata</i> , <i>Phocoena phocoena</i> , <i>Caretta caretta</i>		241 (44%)	0.38

Given the satisfactory data coverage in the entire study zone, no use was made of full coverage spatial distribution predictive models, avoiding interpolation methods whose accuracy could not be assessed. Instead, the information for all the ecosystem components used in this study consisted of direct observations and the datapoints were plotted on a grid of subzones (9 km x 9 km cell size).

We run the analysis on four major components: macrobenthos, birds, demersal fish and marine mammals. The demersal fish component included pelagic species, cephalopods and crustaceans. Sea turtles were not assessed as a separate component, but included in the marine mammals component because of the small size of the reptiles 'dataset and the fact that the underlying observations originated from the same monitoring surveys. Prior to the analysis, general data quality control was applied on all databases. Taxonomy was confirmed using the World Register of Marine Species Taxon match (WoRMS Editorial Board, 2017) to avoid the use of synonymous taxa. Geographical coordinates and dates were standardized for all records.

For the macrobenthos component, the database covered one sampling year and included a total of 145 sites, distributed in perpendicular lines to the coastline, between 13 and 195 m water depth (Martins et al. 2014, 2013). One sediment sample was collected at each site with a 0.1 m<sup>2</sup> Smith–McIntyre grab for macrofauna extraction (sieved on board over 1 mm mesh size) and identified to species level whenever possible, with a total of 26315 animals sampled and 603 species identified.

For the demersal fish component data was used from the 2008 demersal autumn research trawl survey carried out by IPMA (Instituto Português do Mar e Atmosfera) as part of the National Programme for Biological Sampling (PNAB/EC Data Collection Framework). Survey sampling stations were spread along the continental shelf waters, covering depths between 20 and 500 meters. The bottom trawl (14m headline, ground rope with rollers, 20 mm cod-end mesh size) fishing operations were carried out during daylight at an average speed of 3.5 knots, each haul lasting 30 minutes (Chaves 2008). For this exercise, we used the central point of the line survey as a fishing station and the number of individuals per hour of trawl as the abundance index. A total of 88 fishing stations were surveyed distributed in 12 sectors at 3 different depth levels: 20-100 m, 101-200 m and > 200 m, identifying 99 species of fish, 13 of cephalopods, 24 species of crustaceans and 43 species of other groups (echinoderms, cnidarians, bivalves, gastropods, polychaetes, ascidians and nudibranchs).

The birds, marine mammals and sea turtles database was made available by the Portuguese Society for the Study of Birds (SPEA). Sea bird, marine mammal and reptiles census (2004-2012) followed standard European Seabirds at Sea (ESAS) protocols for data collection

(Camphuysen and Garthe, 2004), a standardized ship-based method for recording at-sea distribution of seabirds. It consists of observation units of 5 minutes each, during a continuous route (linear transects), allowing the calculation of animal density estimates for the prospected area (number of animals/ km<sup>2</sup>).

Regarding seabirds, marine mammals and turtles all animals in contact with water within 300 m of the survey transect were counted, and birds in flight were assessed using the snapshot method. More than 19 000 km<sup>2</sup> were surveyed, resulting in more than 200 000 bird observations (belonging to 61 species), 542 marine mammals' sightings (11 species recorded) and 39 observations of sea turtles (1 species recorded). Based on vessel speed and transect width, the surveyed area was calculated and density was estimated as the total number of observed animals divided by the area covered. However, concerning the marine mammal database, some methodological constraints associated with untrained observers might have resulted in species misclassification and in the high proportion of 'non-identified' cetacean records. Also, during ESA dedicated surveys, only one quadrant within 300 m of the survey transect was covered, missing the presence of cetaceans a larger distance from the boat.

### 5.2.3 Marine Biological Valuation protocol

The protocol employed in this study was thoroughly described by Derous et al. (2007c). Within the study area, a set of assessment questions were selected and applied to the different subzones, in order to score them relative to each other. Assessment questions chosen were:

Q1: Is the subzone characterized by high counts of many species?

Q2: Is the abundance of certain species very high in the subzone?

Q3: Is the presence of rare species very high in the subzone?

Q4: Is the abundance of rare species very high in the subzone?

Q5: Is the abundance of ecologically significant species (ESS) high in the subzone?

Q6: Is the species richness (SR) high in the subzone?

Q7: Is the abundance of habitat-forming species (HFS) high in the subzone?

Similarly to Vanden Eede et al. (2014) in a study of the Belgian coast, the marine biological valuation performed in this study was based on the R-script developed by the Flanders Marine Institute (VLIZ), (Deneudt 2013), adapted to the available biological data. The assessment questions were based on the criteria of rarity, aggregation and fitness consequences and transformed into mathematical algorithms (see Supplementary information table S.1 for full description of assessment questions, valuation criteria and algorithm description) and applied

to each ecosystem component dataset separately. This resulted in a numerical output further scored into a semi-quantitative classification of five classes (1-5). In each subzone, the total scores for all assessment questions were added per ecosystem component (each assessment question having an equal weight over the total score) resulting in a biological value (BV) score per subzone. The ecologically significant species and habitat forming species chosen are listed in Table 5.1 and were selected based on expert knowledge and/or based on the DEVOTES Keystone Catalogue, which is a review of potential keystone species of the different European marine habitats (Smith et al. 2014). The total BV was calculated for each subzone by averaging the values of the various ecosystem components (when there was only one ecosystem component, the total value assumed its score) and classified into a five value scoring system: 1 = Very Low, 2 = Low, 3 = Medium, 4 = High, 5 = Very High. These scores were displayed on colour graduated BV maps. The correlation between each component and the total BV scores was measured by calculating the Spearman correlation.

Data availability values were determined by the number of samples (/observations) of each component taken (/made) in each subzone. It was calculated for each ecosystem component and for all components together, and divided into a three value scoring system: 1 = Low, 2 = Medium, 3 = High. The reliability indices scored how many assessment questions were answered per subzone, compared to the total number of possible questions. A reliability valuation map (scoring 1 = Low, 2 = Medium, 3 = High) was created for each component and for all components together. It displays the “trustworthiness” of the data, and thus the value of subzones with less available data for all ecosystem components are scored as being less reliable than subzones valued on all the ecosystem components. This information should be consulted and discussed together with the BV map for a better interpretation of the overall results.

#### **5.2.4 Hotspot identification**

The Hotspot spatial statistics analysis (Getis-Ord Gi\*) was run in ArcMAP 10.1 to spatially cluster subzones with either significant high or low values. This tool identifies hotspots by examining each subzone within the context of neighboring elements (Getis and Ord, 1992), evaluating the spatial association of a variable within a specified fixed distance band of a single point (in this case, the geometric centroid of each grid cell). In this sense, isolated large value cells were considered as outliers. We set up the distance threshold so as to include three neighbors of a grid cell. The result is a map of standardized z-scores reflecting the average BV within the defined radius relative to the whole domain, which can be compared to expected values under a normal distribution. Setting a confidence level of 95% delimits areas of spatial

significance at z-values +1.96 standard deviations from the mean in the case of hotspots, and -1.96 standard deviations from the mean in the case of coldspots.

### 5.2.5 Spatial overlap

#### 5.2.5.1 Conservation areas

We investigated the spatial overlap of the total biological value obtained in this study with Natura 2000 marine conservation areas. This European network of nature protection is composed of sites designated under the Birds Directive (Special Protection Areas, SPAs) and the Habitats Directive (Sites of Community Importance, SCIs and Special Areas of Conservation, SACs). While SPAs are designated directly by Member States, SCIs are first proposed by Member States and, when approved by the European Commission, are designated as SACs. Here, we compare our results with the recently expanded marine SPAs and with the formalized proposal for the creation and expansion of marine SCIs, which await the approval by competent national authorities. Some already designated SCIs cover coastal areas, but are essential littoral land sites covering a narrow strip of marine area of up to 20m deep, and will not be considered here. For full illustration of Natura 2000 Network SPAs and SCIs in continental Portugal see Supplementary information Figure S.1.

SPAs place great emphasis on the protection of habitats for endangered and migratory species and member states should identify and delimit the areas so as to ensure that all 'most suitable territories' are designated for protection, based on scientific knowledge. In Portugal, 7 SPAs which incorporate marine areas comprise 26% of the continental shelf area (6188 km<sup>2</sup>): Ria de Aveiro, Aveiro/Nazaré, Ilhas Berlengas, Cabo Raso, Cabo Espichel, Costa Sudoeste and Ria Formosa. These have been created and recently expanded, based on the available information of occurrence, distribution and reproduction of numerous seabird species. Geographic Information System layers for N2000s were obtained from the Portuguese ICNF (Institute for Conservation of Nature and Forest). The spatial overlap analysis was performed using the ArcGIS software (ESRI, 2006). The polygons corresponding to N2000 SPAs were used to quantify the area (in km<sup>2</sup>) overlapping the different subzone BV scores. Finally we overlapped the total BV Hotspots with the current marine SPAs and proposed SCIs. We used the SCIs marine polygons included in the technical proposal recently submitted by the national nature and biodiversity conservation authority to extend the Habitats Directive to the marine environment (Maceda-Praia da Vieira, Costa de Setúbal and Costa Sudoeste).

### 5.2.5.2 Habitat maps

Lastly, we used the EUSeaMap broad-scale seabed habitat maps (available at [www.emodnet-seabedhabitats.eu](http://www.emodnet-seabedhabitats.eu), see Fig. 5.7A and Fig. 5.7B) to analyze the association between the total valuation outputs with local physical characteristics. EUSeaMap—Mapping European seabed habitats (Populus et al. 2017) is a broad-scale modeled habitat map covering over 2 million km<sup>2</sup> of European seabed (available in a polygon format layer), built in the framework of MESH (Mapping European Seabed Habitats). Habitats were classified according to EUNIS (European Nature Information System) classification system which provides a common and comparable European reference set of habitat types: "rock", "coarse sediment", "mixed sediment", "sand", "muddy sand", "sandy mud" and "mud". In addition, we used the biological zonation (EUSeaMap) for habitat characterization, based on a vertical zonation scheme reflecting changing conditions of light penetration/attenuation and disturbance of the seabed by wave action: the infralittoral, the circalittoral, the deep circalittoral and the upper slope. The infralittoral zone extends from the intertidal seafloor to a boundary marking the end of favorable light conditions for the growth of seagrass and green algae. The circalittoral zone extends to a maximum depth at which the seabed is influenced by waves (where depth is  $\leq \frac{1}{2}$  wavelength) and the deep infralittoral and upper slope expand to a maximum depth of 200 m and 750 m respectively. Independent one-way ANOVAs, followed by post-hoc Tukey tests, were performed to test any effect of each factor (substrate type and biological zone) on total BV.

## 5.3 Results

### 5.3.1 Biological Value (BV) and Hotspots classification

The BV maps for each assessment question, data availability and reliability indices per ecosystem components can be seen in Supplementary information Figures S.2-S.5. When looking at total data distribution (all components together), there were 546 subzones with data (covering an area of 41866.5 km<sup>2</sup>). The bird component contributed with the highest amount of data for the total valuation, followed by the marine mammals and turtles, macrobenthos and finally the demersal fish component (with 534, 241, 115 and 86 subzones with data, respectively, Table 5.1). The great majority of the data (70%) was concentrated within continental shelf waters up to 200 m. Total BV maps and hotspot analysis per ecosystem component are illustrated in Fig. 5.2.

The valuation map for the bird component (Fig. 5.2A) clearly shows the high ornithological BV of the entire Portuguese coastal zone. High and very high values were distributed along the



coast, mainly at less than 100 m depth in the north and center and up to 200 m depth in the south. In contrast, the southwest coast is characterized by very low to medium values up to the region around Cabo São Vicente, where high values appear again. The hotspot map for the bird BV scores (Fig. 5.2E) visibly shows this discontinuity of higher values along the southwest coast.

For the demersal fish component, high and very high BV were located mostly outside Aveiro estuary, around the isolines for 100-200m water depth, and in the southwest at deeper depths of around 300 m. However, most of the high and very high BV was concentrated in the south region between 100-200 m (Fig. 5.2B and Fig. 5.2F). Sampling effort in 2008 was identical for the entire study area, and data availability depended on the location of the 88 trawled stations.

For the macrobenthos, sampling stations were evenly distributed along the west coast of Portugal but placed in closer proximity in the south coast. The valuation and hotspot map show a distribution of higher valuable areas off Aveiro, Cabo Carvoeiro, south from Setubal bay and in the south region (Fig. 5.2C and Fig. 5.2G respectively).

The marine mammals 'component only showed very high BV in the southern region, at a depth of 100-200 m, around São Vicente cape in the west, and near the Spanish border in the east (Fig.5.2D and Fig. 5.2H). High valuable areas were located in the north, around Aveiro region within less than 100m depth and along the continental slope. Other high valuable areas for this component were present at a shallower depth around Cabo Raso and dispersed around the southwestern and southern region at the continental edge.

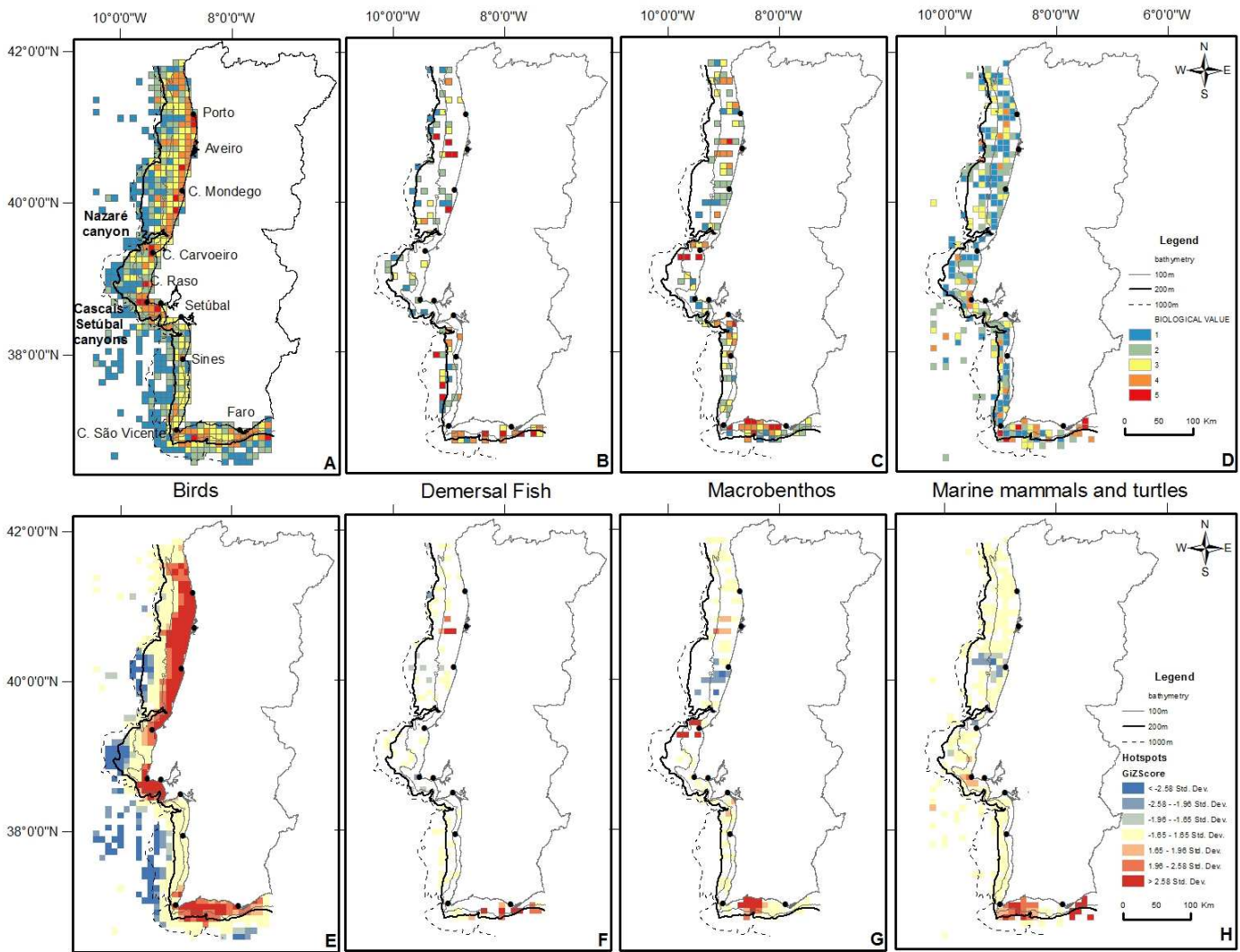


Figure 5.2 Biological valuation maps for each ecosystem component: A birds, B demersal fish, C macrobenthos, D marine mammals and turtles (with common legend) scored into a five value scoring system: 1 = Very Low, 2 = Low, 3 = Medium, 4 = High, 5 = Very High. Hotspot classification for each ecosystem component: E birds, F demersal fish, G macrobenthos, H marine mammals and turtles (with common legend) showing z-scores using 95% confidence levels to determine the areas of spatial significance.

The map of total data availability (Fig. 5.3A), which measures the number of observations/samples in each subzone, shows a quite homogeneous distribution in the study area, with the great majority (96%) of the grid cells containing the same magnitude of available data. Even though data reliability per ecosystem component was very high for the great majority of subzones with data (Supplementary information, Figures S.2-S.5), the different coverage and sampling effort of the datasets caused the reliability (proportion of assessment questions that could be answered by subzone) of the total BV (Fig. 5.3B) to oscillate between low (%48), medium (%37) and high (%15). The Total BV map for the whole study area is shown

in Fig. 5.3C. Very low, low, medium, high and very high value areas covered 36%, 35%, 18%, 10% and 1% of the study area respectively. Notably, most of the higher BV scores were consistently located near the coastal zone, in shallower areas. In fact, low and very low values cover 90% of the total study area comprised zones of higher bathymetry ( $> 100$  m). When we look at the results within less than 100m depth, high and very high BV cover almost 25% of the area, dispersed along the coast, with predominance in the north, center and south regions. The hotspot analysis for the total BV identified four main hotspot zones of significantly high biological value inside the continental shelf waters; off Aveiro and expanding to the north, off Cabo Carvoeiro, the region off Cabo Raso and Setúbal bay up to Arrábida bay, and covering the majority of the south region (Fig. 5.3D).

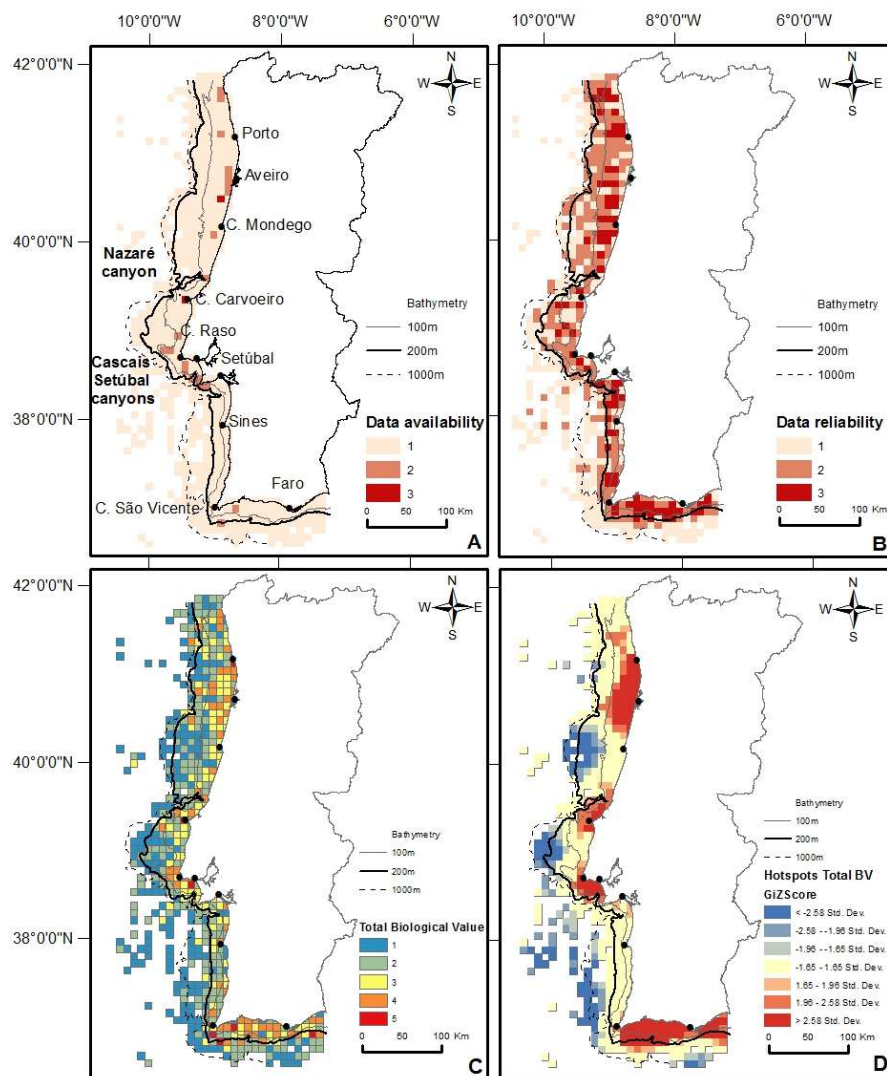


Figure 5.3 **A** Total data availability scores (1 = Low, 2 = Medium, 3 = High), **B** Total data reliability scores (1 = Low, 2 = Medium, 3 = High), **C** Total biological value (1 = Very Low, 2 = Low, 3 = Medium, 4 = High, 5 = Very High). **D** Hotspot classification showing z-scores using 95% confidence levels to determine areas of spatial significance.

When matching up the reliability indices with the total BV, we found that 70% of the lowest BV, 22% of the high and 38% of the highest total BV have low reliability (Fig. 5.4). This is caused when the scored subzones comprise information from only one component (out of 4). However, it is important to notice that reliability was higher in coastal areas; in areas shallower than 100 m depth, medium and high reliability scores covered 43% and 33% of the area respectively.

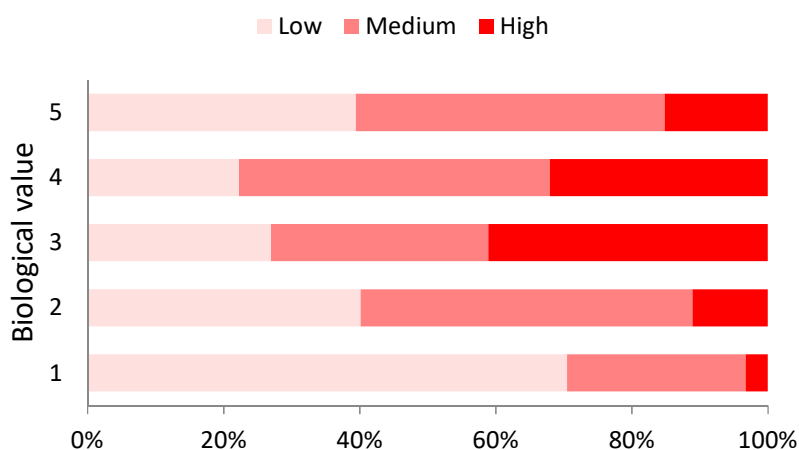


Figure 5.4 Reliability of the total BV scores.

Spearman coefficient of determination ( $r^2$ ) demonstrated the magnitude of the association between individual components BV and the total BV. As expected, each component's score was significantly positively correlated with the total score. The bird component, which delivered the highest amount of data for the analysis, explained most of the trends detected in the total BV scores contributing to 64% of the variation in the total scores, followed by macrobenthos (21%), mammals (15%) and fish (5%) (Table 5.1).

### 5.3.2 Spatial Overlaps

#### 5.3.2.1 – Conservation areas

Marine SPAs in mainland Portuguese continental waters are illustrated in Fig. 5.5A. The spatial overlap of the total BV with the marine fraction of the SPAs can be seen in Fig. 5.5B. 3% of the total area of very low, 16% of the low, 29% of the medium, 28% of the high and 20% of very high total BV are contained inside currently designated SPAs. Concerning individual SPAs, the percentage coverage of total BV can be seen in Fig. 5.6. Very high BV areas were only included in Costa Sudoeste, Cabo Raso and Ilhas Berlengas and with very low percentage (3-5% Fig. 5.6). Ria de Aveiro was the SPA with the largest percentage of high BV areas included (52%) followed by Cabo Raso, Ilhas Berlengas, Costa Sudoeste, Aveiro/Nazaré and Ría Formosa

(25, 18, 18, 16 and 15 % respectively, Fig. 5.6). Cabo Espichel included only low and medium values (18 and 82% respectively), and in all the SPAs but Cabo Espichel and Aveiro low and very low values make up more than 40% of the area protected (40-60%, Fig.5.6). Fig. 5.5C shows the spatial overlap of the hotspot analysis for the total BV and the SPAs and proposed SCIs. It shows that the two BV hotspot areas located in the central region are totally included inside the Ilhas Berlengas and Cabo Raso SPAs. The hotspot around Aveiro expands much further beyond the Ria de Aveiro and Aveiro/Nazaré SPA, being overlapped with the northern part of the proposed Maceda-Praia da Vieira SCI. The hotspot located in the southern region is outside any designated SPA with very limited overlap with Costa Sudoeste SPA around Cabo São Vicente. Yet, the proposed SCI of Costa Sudoeste does cover an important area of the west side of the southerly BV hotspot but the easternmost part falls outside any designated or proposed conservation area.

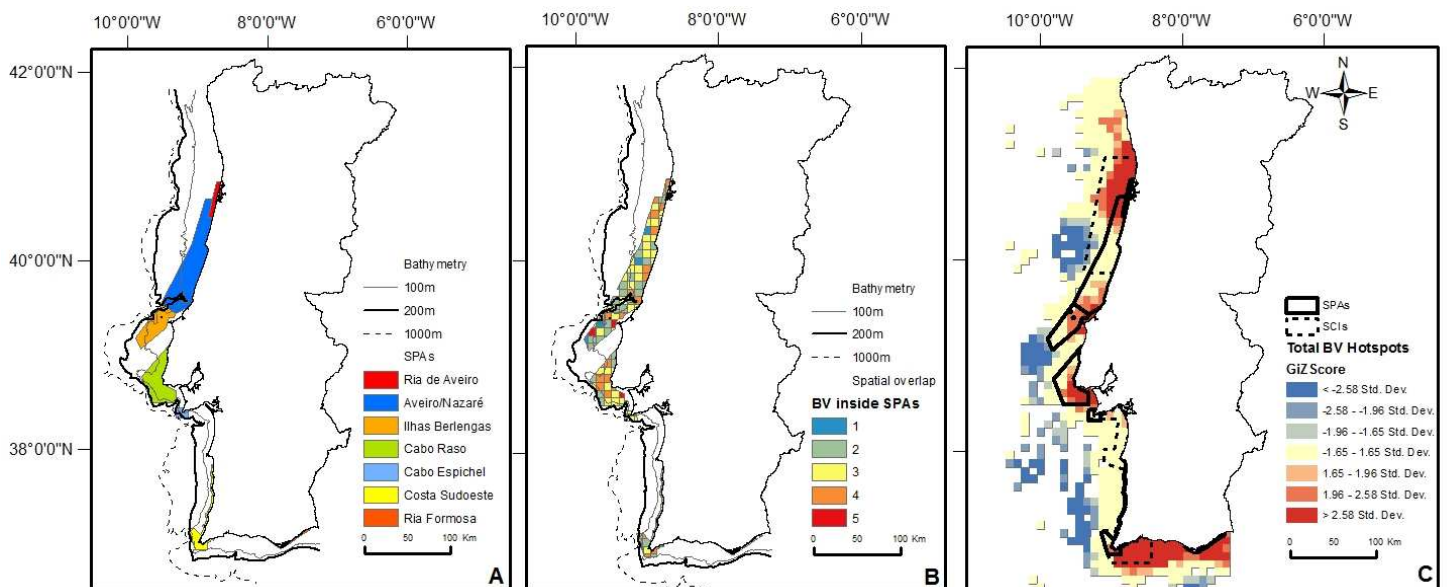


Figure 5.5 **A** Marine Special Protected Areas (SPAs): Ria de Aveiro, Aveiro-Nazaré, Ilhas Berlengas, Cabo Raso, Cabo Espichel, Costa Sudoeste and Ria Formosa. **B** Spatial overlap of the total BV with SPAs. **C** Spatial overlap of the total BV hotspot analysis with SPAs and recently proposed marine Sites of Community Importance (SCIs, from north to south: Maceda-Praia da Vieira, Costa de Setúbal and Costa Sudoeste).

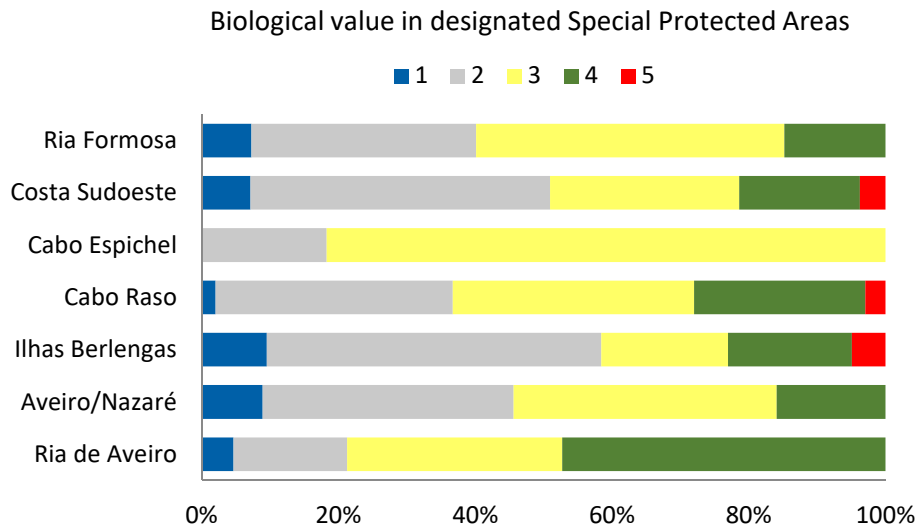


Figure 5.6 Stacked graph illustrating the total biological value within Portuguese continental marine Special Protected Areas (SPAs).

#### 5.3.2.2–Habitat maps

The EUSeaMap broad-scale seabed substrate map (Fig. 5.7A) and biological zone (Fig. 5.7B) were selected for this analysis. The spatial overlap of the total BV and substrate map resulted in each subzone being defined by a predominant substrate type and biological zone (in terms of total grid cell area). The substrate type was responsible for significant differences in the total BV ( $F = 3.104$ ,  $p < 0.0001$ , Fig. 5.8), with a gradient on BV values from coarser to fine sediments. Regarding the biological zone, we analyzed both individual components and the total BV (Fig. 5.9). For the total BV, higher scores were found in the infralittoral and circalittoral, when compared to deep circalittoral and upper slope ( $F = 25.180$ ,  $p < 0.0001$ ). This trend was observed in all components, with some deviations, although significant differences were only found in the birds BV ( $F = 28.214$ ,  $p < 0.001$ ) and macrobenthos BV ( $F = 3.193$ ,  $p < 0.05$ ).

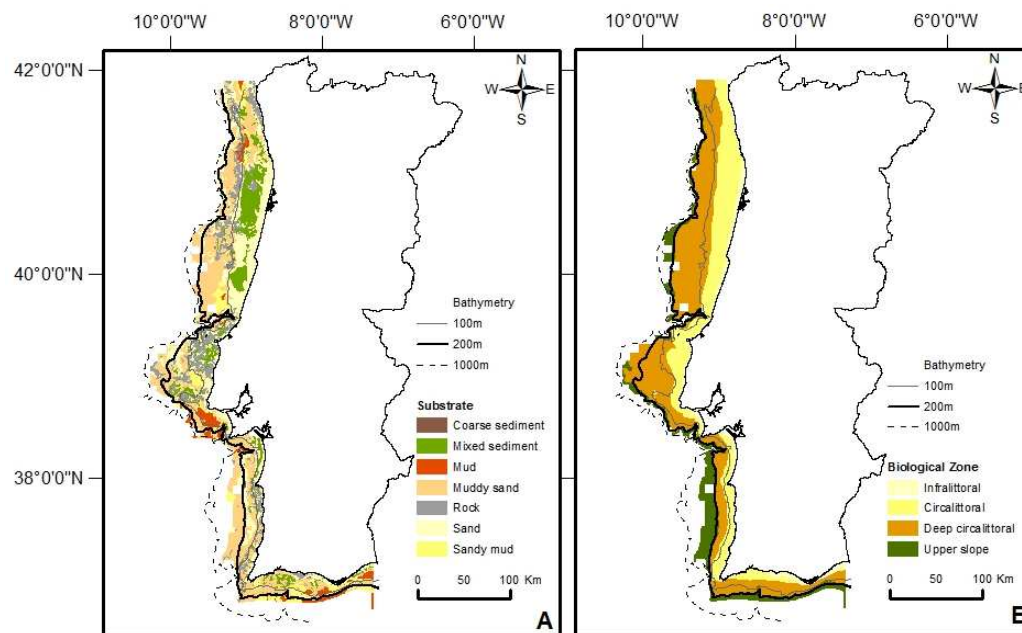


Figure 5.7 EUSeaMap broad-scale seabed habitat maps for the Portuguese continental shelf waters: **A)** Substrate type layer and **B)** Biological zone layer.

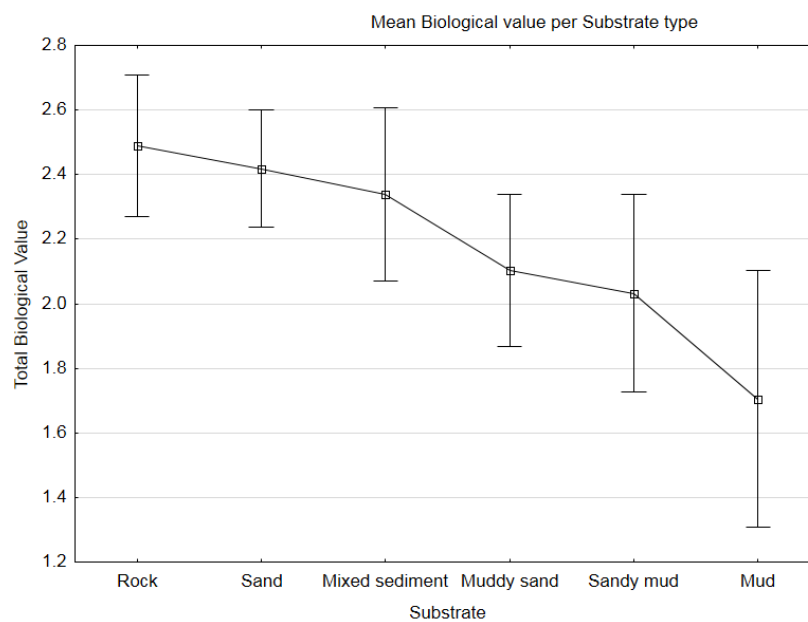


Figure 5.8 Mean total BV per substrate type. Bars represent means  $\pm 0.95$  Confidence interval. Letters above bars indicate homologous groups after a Tukey HSD test ( $p < 0.05$ ).



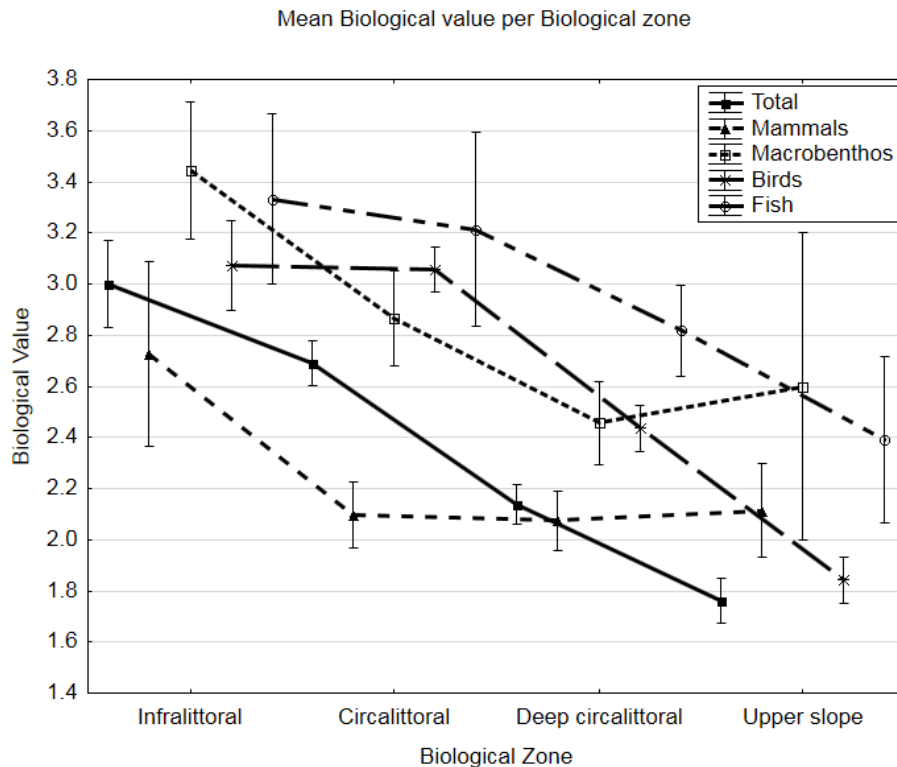


Figure 5.9 Mean total BV per biological zone. Bars represent means  $\pm 0.95$  Confidence interval. Letters above Total BV bar indicate homologous groups after a Tukey HSD test ( $p < 0.05$ ).

## 5.4 Discussion

The biological valuation presented here described patterns of biological value consistent with the physical and biological oceanography and local hydrodynamics of the continental Portuguese coast. This study not only confirms and matches previously identified valuable areas for protection (especially in the northern and central regions, around Aveiro, Cabo Carvoeiro, and Cabo Raso), but also highlights the value of currently unprotected sites, mainly north of Aveiro and in the southern region.

### 5.4.1 BV per ecosystem component

The bird component BV hotspot map showed significantly valuable areas in the southern region and along the western coast, mostly in the widest parts of the continental shelf (situated in the northern and central sector). This area is strongly influenced by seasonal upwelling patterns and high productive waters, determined by the bathymetry, coastal morphology, and local wind conditions (Relvas et al. 2007). In Portugal, marine important bird areas (IBAs) have been defined based on seaward extensions of breeding colonies, non-breeding coastal concentrations and migration bottlenecks (Ramirez et al. 2008). The IBAs results, which also highlight the importance of the northern and central coast (Ria de Aveiro,



Aveiro/Nazaré, Berlengas, Cabo Raso and Cabo Espichel), has been the basis to the creation and extension of current SPAs. Recently, Araújo et al. (2017) underlined the importance of the northern and central sectors of Portuguese shelf to the critically endangered Balearic shearwater *Puffinus mauretanicus*, which was chosen in our analysis as an ecologically significant species. Our analysis further demonstrated the importance of the southern region for the birds BV, particularly in the widest portion of the shelf, situated between Cabo SãoVicente and Faro.

For the macrobenthos component, BV scores showed a heterogeneous gradient along the shelf, with high BV areas found off the Aveiro, around Cabo Carvoeiro, south from Setubal bay and in the south region, with a hotspot around the Cabo Carvoeiro and Berlengas area, and in the southern region. An analysis of the diversity and spatial distribution patterns of the soft-bottom macrofauna communities on the Portuguese continental shelf using the same macrobenthos dataset also exposed these locations as having high macrofauna abundance, high alpha and Shannon–Wiener diversity and high Pielou evenness indices (Martins et al. 2013). The authors identified depth range, hydrodynamic regime, sediment grain-size and total organic matter content as the variables which best related to the macrofauna distribution patterns.

Highest BV for the demersal fish component were found in the water depths of around 100–200 m in the north shelf, in the southwest at depths of around 300 m and in the south between 100–200 m. Differences in groundfish species assemblages have been observed in other studies, showing a north–south biological discontinuity related to shelf bathymetry, coastal morphology and oceanography along the northern and southern parts of the shelf (Gomes et al. 2001, Sousa et al. 2005). Similar to Sousa et al. (2006), based on an analysis of a ten year groundfish survey on the Portuguese shelf and upper slope, we found lower species richness to the north and higher to the south (see Supplementary information Figure S.3 –F). However, similar to the macrobenthos BV results, there was generally high variability and patchy distribution in demersal fish BV scores along the study area. This is probably the result of two main factors. Firstly, the complex topography of the continental shelf and the heterogeneous distribution of substrate types (Martins. et al. 2012), which is known to influence the structure and diversity of benthic species assemblages. Sediment grain size is mostly related to differences in the continental shelf morphology and hydrodynamic features, the location and extent of rivers, leading to variation and patchiness in the benthic community. Secondly, there was a clear limitation in the spatial and temporal resolution of the available macrobenthos and demersal fish database. Although survey sampling had a reasonable coverage along the whole study area, single-year databases do not reflect inter-annual and

seasonal changes and thus too short to draw safe conclusions about biological value patterns. For this reason, it is possible that some BV scores may be an artifact due to insufficient sampling in the area and it will take greater sampling intensity, both temporally and spatially, to detect more consistent trends in species distributions, and therefore also in the identified local biological value.

The marine mammals' component which also included a limited number of the sea turtles observations only showed very high BV in the southern region, at a depth of 100-200 m, around São Vicente cape in the west, and near the Spanish border in the east. Some high valuable areas were located off Aveiro. Other high valuable areas were situated at a shallower depth around Cabo Raso and patchily scattered around the southwestern and southern region at the continental edge.

Although several institutions are currently monitoring the marine mammal population along the coastal and oceanic waters, there is limited information on the overall distribution along the mainland Portuguese shelf waters as most studies focus on localized surveys on species occurrence, distribution and interaction with fisheries (Brito Cristina et al. 2009, Martinho et al. 2015). In these studies, the distribution of dolphinids along the Portuguese central west coast was mainly linked with topographic features such as sheltered bays, submarine canyons and major estuaries, which drive highly productive surface water and input of nutrients. The southern region, which also showed the highest marine mammal's BV values in our study, has already been recognized important for cetaceans (Castro et al. 2013) and specifically for the presence of baleen whales (Laborde et al. 2015).

Ongoing studies, such as the annual aerial campaigns developed within the Life+ MARPRO project (LIFE09 NAT/PT/000038) constitute the first standardized dedicated effort to assess large scale marine mammal abundance and distribution for the entire Portuguese Exclusive Economic Zone. These efforts greatly improve the quantity and quality of sighting records, overcoming the methodological constrain described for the marine mammal database used here. Also, it allows for the evaluation and monitoring of the abundance, occurrence and health of marine mammal's populations, to update current national databases and policies and to revise the protocol applied here.

#### **5.4.2 Total BV and biodiversity hotspots**

The total BV results showed higher scores consistently located near the coastal zone. Regarding data availability, and despite the study area showing relatively low availability, the map showed a quite homogeneous distribution of scores, with higher data availability scattered in some coastal grid cells in the northern and central regions. This means that most

grid cell scores were based on low number of samples/observations for each ecosystem component, highlighting the need to increase sampling coverage during national monitoring surveys. Data reliability showed lower scores mainly outside the 200 m bathymetric zone in the western coast and higher values at the coastal fringe and most of the south region up to 200 m. High reliability scores indicate high number of ecosystem components in each grid cell analyzed and reduces subjectivity of the total result. Off the 200 m depth limit, the valuation was less reliable, as the bird component dominated the data both spatially and temporally, and the BV of birds alone mostly explained the observed trends of the total BV. High and very high BV and medium to high reliability characterized the coastal area up to 100 m depth. Very high and high BV appeared mostly in the northern, center and southern regions.

The hotspot analysis identified four main areas: stretching to the north and south off Aveiro, near Cabo Carvoeiro, south of Cabo Raso, and covering the majority of the southern region. While there are regional and national studies confirming the importance of these areas for individual ecosystem components as aforementioned, there are no published evidences at national scale on marine biodiversity patterns using a wide range of taxonomic groups. The hotspot approach used here does not discard other areas in need of protection, but it may help in setting priorities to define crucial areas in conservation strategies for diverse global biota (Myers et al. 2000).

The hotspots areas identified in this study seem to be related with large-scale topographic and oceanographic characteristics combined with mesoscale features, which influence biodiversity and affect the dynamics of the whole ecosystem. The heterogeneous coastline orientation, prominent capes, submarine canyons, large estuaries and river discharges, interacting with mesoscale features, such as fronts, buoyant plumes, eddies, stratification and wind-induced upwelling areas, result in complex water circulation and seasonal high productivity (see Relvas et al. 2007 for a review on the physical oceanography of the western Iberia ecosystem). In the west coast, these features are particularly important in the northern and central zone, where the northerly winds are more stable and the wide and lower shelf results in a more persistent and homogeneous upwelling. This fact might explain the higher BV in the northern and central area, when compared with the southwestern sector.

A positive BV gradient was found from muddy to rocky substrates, showing substrate type as an important factor for the BV distribution. Habitat complexity and sediment types have been referred as physical surrogates for biodiversity patterns (Smith et al. 2009). We also detected higher BV found in the infra and circalittoral biological zones, reflecting a depth gradient in the BV over the study area. The coastal areas were associated with the highest BV, similar to

previous studies applying the same protocol (Deros et al. 2007c, Pascual et al. 2011, Vanden Eede et al. 2014).

#### **5.4.3 Limitations and opportunities**

Total data availability was estimated as low in most of the study area, meaning a limited number of observations/samples per grid cell. This constraint was particularly restrictive for the relatively immobile macrobenthos component, as the entire grid cell was characterized by a single 0.1 m<sup>2</sup> grab sample. Although the grid cell size might represent a good compromise for mobile components, that is not the case for less mobile and sessile benthic fauna. The use of smaller grid cells for such components would be more representative of the associated habitat and together with greater spatial sampling efforts, would stand for more realistic BV of the benthic communities, and consequently total BV patterns.

The temporal scale limitation, as mentioned earlier for the macrobenthos and demersal fish component, is also of great importance, since one year databases can not reflect the inter-annual and seasonal differences which characterize biological systems, particularly in upwelling areas. So, it is important to recognize that we have applied this protocol given the accessible national biological datasets with sufficient spatial coverage and sampling effort at the time of this study and our analysis should be revised and updated as new relevant data becomes available.

The addition of spatial data on the distribution and abundance of other important marine ecosystem components, such as pelagic fish, phytoplankton and zooplankton will be crucial to uncover key patterns in the water column and the surface waters. Qualitative and quantitative studies of the phytoplankton distribution and abundance on the Portuguese continental shelf revealed strong seasonal variability at regional and local scales, mainly related to water column stratification, nutrient availability and intensity and persistence of upwelling conditions (Moita 2007).

Also, given the size of the subzones and nature of the databases, our results fail to provide a complete analysis of the important biological communities at the intertidal and shallow subtidal coastal zones, composed of valuable habitat-forming and engineering species. For this reason, it would be important to repeat this exercise at a smaller spatial scale, including different habitats, such as transitional waters, seagrass and kelp beds, saltmarshes, rocky and sandy shores to improve the valuation at the coastal area and capture the structure and function of littoral ecosystems. While most data were simply not available, other could not be used due to insufficient spatial coverage and/or lack of abundance information, which could create bias in the total BV calculation, underestimating BV due to lack of information.

The flexibility and easy adjustments to the protocol permitted the remodeling of algorithms to include local knowledge on ecosystem components, as well as spatial comparisons with other available environmental databases. Moreover, the set of assessment questions can be adapted to different processes and organizational levels of biodiversity as proposals for new valuation criteria emerge. This way, the method allows for future refinement in the choice of biological-based metrics to define the different facets and dimensions of biological systems, such as the ones recently appointed in the Essential Biodiversity Variables (EBVs) framework: genetic composition, species populations, species traits, community composition, ecosystem structure and ecosystem function (Pereira et al. 2013).

#### **5.4.4 Overlap with conservation Areas**

This study shows that there is a good agreement between the spatial coverage of high BV and hotspots with the continental Portuguese SPAs. Also, it shows that the proposed SCIs can complement the protection status of valuable areas. Even though the SPAs have been designated to safeguard the habitats of migratory and threatened birds under the Birds Directive, it is relevant to compare its location with our integrative biological hotspots. Being important top predators, seabirds have been described as good indicators of the health of the marine environment, as they travel or forage in productive marine hotspots (Parsons et al. 2008). This way, at-sea distributions of seabirds can act as effective proxies for identifying priority sites for conservation of data-deficient marine species (Harris et al. 2007, Hooker and Gerber 2004). This is significant since the SPAs management plans should not only guarantee the conservation of the habitats and species for which they were designated but also manage activities to be developed within its boundaries, requiring a favorable opinion of the national environmental management authority, and potential impact assessment (Decree-Law 140/99). Almost half of the total area containing high and very high BV fell inside currently designated SPAs. Also, the biodiversity hotspots around Cabo Carvoeiro and Cabo Raso were included inside the Ilhas Berlengas and Cabo Raso SPAs, respectively. The Aveiro BV hotspot is partially integrated in the northern SPA, but is fully included in the northern part of the proposed SCI of Maceda-Praia da Vieira. The main spatial disagreement was observed in the southern region, which showed very high BV scores for all ecosystem components separately and for total BV, but is currently under little protection status. At present, the only designated protected area in the south region is the area surrounding the Cabo São Vicente and the Ria Formosa SPA and Natural Park comprising an inter-tidal meadow lagoon, with very limited coverage of coastal and deeper habitats. The proposed SCI of Costa Sudoeste covers an important area at the

westernmost side of the southern hotspot, leaving the east side under no current or proposed conservation status.

In this way, our study supports the location of existing SPAs and proposed SCIs as important sites for the conservation of valuable areas and suggests the need to extend the protection along the southern region. Furthermore, management plans should establish structured and evidence-based instruments to guide managers and assessing authorities to make sound decisions in accordance with the ecological needs and conservation of vulnerable habitat types and the species.

#### **5.4.5 Management Implications**

Identifying world-wide patterns and trends in marine biodiversity using several ecosystem components is important for conservation biology (Tittensor et al. 2010), assisting the spatial priority setting for biodiversity sustainability and the challenges posed by ecosystem-based MSP processes (Gilliland et al. 2008).

At the European level, the MSFD directive refers to biodiversity as a key indicator to achieve “Good Environmental Status”, by stating “the quality and occurrence of habitats and the distribution and abundance of species should be in line with prevailing physiographic, geographic and climatic conditions”. Recently, Portugal has been used as a case study using the Nested Environmental status Assessment Tool (NEAT), a large scale marine biodiversity assessment under the MSFD. The study used national data on several ecosystem components and the overall results exposed Portugal with a “Moderate” environmental status (on a scale of 5, from Poor to High) (Uusitalo et al. 2016). Importantly, it also adverted for major knowledge gaps in species distribution and areal coverage.

At the national level, Portugal has already developed an initial assessment of the current environmental status of national marine waters with a comprehensive biological characterization of marine waters under the national jurisdiction (MAMAOT 2012). It was based on the marine biological valuation protocol and covered broad evaluation areas up to 200 nm using data on phytoplankton, zooplankton, macrobenthos, bivalves, cephalopods, crustaceans, fish, birds and mammals. Although this assessment initiative analyzed each component separately, it did not generate a total BV map across components. This report concluded on a “good environmental status” for the major habitats (coastal and pelagic) and for the majority of the functional groups analyzed.

Even though these general studies are crucial to attend to international policy demands, the scanty spatial resolutions of the results are a major limiting factor when dealing with the imminent pressure and impacts of local maritime exploitation. The rise of the blue growth

economy is rushing countries to make smaller scale decisions on the spatial allocation of maritime human activities. In this regard, the marine biological valuation tool presented here represents a clear advantage in relation with the MSFD approaches in terms of spatial resolution of the environmental metrics. Instead of providing a single “status” for major habitats, ecosystems components and biodiversity, it provides a multi-metric ecological indicator, with a relative scoring system of intrinsic biological value over small scale subzones over the entire study area.

In Portugal, the legally binding MSP is responsible for dealing with the growing and competing demands for maritime space, such as oil and gas exploration, fisheries, seabed mining, maritime shipping, aquaculture, coastal and maritime tourism, marine biotechnology, ocean energy and environmental protection. A recent study by Fernandes et al. (2017) showed that the continental Portuguese coastal space is experiencing high cumulative impacts caused by current activities and uses, and alerted for the need to improve environmental assessment tools. Interestingly, all the hotspots for the total BV detected in our study coincide with areas where anthropogenic impacts (mainly fisheries and pollution) were also greater. The authors also alerted for the fact that nature conservation areas considered in the ongoing MSP plan (INAG 2012) were still prone to exploration, such as fishing, aquaculture, oil, wave and offshore wind inspection or sand and gravel extraction. The environmental section of the plan further states that “the information currently available to assess marine ecosystems and biodiversity as well as the cultural values associated with the sea is scarce and fragmented”. Knowledge gaps are identified as one of the main obstacles to the implementation of the operational aspects of the program. Consequently, if marine policies are not built upon scientifically-recognized ecological principles on the processes and functioning of biological communities, the ecosystem based approach underlying MSP policies might be compromised. In this sense, biological valuation maps can highlight valuable areas useful within the scope of MSP. Also, it allows for the integration of biodiversity with socio-economic and best expert judgment criteria to assist in space-use conflicts in an appropriate spatial scale.

This study has proved useful to outline the importance of not only good sampling strategies along coastal and continental shelves, but also the significance of offering scientists the opportunity to access and link scattered data for informative biological valuations, essential to assist science reproducibility and to minimize biases in policy development. In this sense, we advocate for the need to have environmental researchers, computer scientists and policy makers working together on the creation and maintenance of a national marine biodiversity database. Centralized and up-to-date information on the distribution and abundance of marine organisms and habitats is crucial to uncover processes driving biodiversity and to assess

biodiversity trends against environmental variability. Finally, this approach should stimulate discussion among Portuguese scientists, stakeholders and managers involved in the Natura 2000 network, MSFD and MSP process on value-based criteria to define areas of biological importance to safeguard environmental sustainability in “an ocean of opportunities”.

## 5.5 Conclusions

The application of the marine biological valuation and hotspot analysis to the Portuguese continental shelf waters resulted in the recognition of four major biologically valuable regions, despite temporal and spatial data limitation. These areas matched topographic and physical oceanographic attributes known to influence biodiversity, such as coastline orientation, prominent capes, submarine canyons, large estuaries, habitat type and wind-induced upwelling areas. The hotspots fall within the boundaries of Natura 2000 designated SPAs and proposed SCIs, except in the easternmost part of the southern hotspot. Quantitative-based approaches such as the one presented here may assist in guiding management plans and decisions to safeguard local biological value and defining priority areas for conservation at the scale of tens of kilometers, useful within the scope of MSP.

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### 5.7 Supplementary Information - Assessment questions, data availability, data reliability and biological value scores

**Table S.1** – Algorithms used in the R script for the assessment questions, data availability, data reliability and biological value scores (adapted from Eede et al 2014).

Assessment question	Valuation criteria	Ecosystem components	Algorithm
<b>Q1: Is the subzone characterized by high counts of many species?</b>	<b>Aggregation/fitness consequences <sup>1</sup></b>	macrobenthos birds demersal fish marine mammals (All components)	Determine the species which are regularly occurring in your study area, selecting all the species which occur in more than 5 % of your records The average density was calculated per grid cell (subzone) for every regularly occurring species Create 5 density classes with values between 1 and 5 (per species). Assign values to data for all species and sum the values in every grid cell Divide the resulting summed values again in 5 classes (based on the range of the values).
<b>Q2: Is the abundance of certain species very high in the subzone?</b>	<b>Aggregation/fitness consequences <sup>1</sup></b>	All components	Determine the species which are regularly occurring in your study area by selecting all species which occur in more than 5 % of the subzones Determine the mean density of every species for the whole study area (=X) Calculate the mean density of every species for every subzone (=Xi). Calculate the ratio Xi/X for every species in each subzone Determine the 5 % subzones with the highest ratio. Calculate the percentage of the density of every species that occurs in the 5 % most important subzones (=Y) Determine in how many subzones every species occurs (=Z). Calculate the ratio Y/Z which is the aggregation coefficient for each species. Multiply the ratio Y/Z with the ratio Xi/X and divide these values in 5 classes with values between 1 and 5.
<b>Q3 and Q4: Is the presence/abundance of rare species very high in the subzone?</b>	<b>Rarity <sup>2</sup></b>	All components	Determine the species which occur in less than 5% of your subzones (rare species). Interpolate presence or density data of species to the chosen subzones. Create 5 presence or density classes with values between 1 and 5 (with an equal amount of subzones in each class). Assign values to data for all species and sum the values in every subzone. Divide the resulting summed values again in 5 classes.

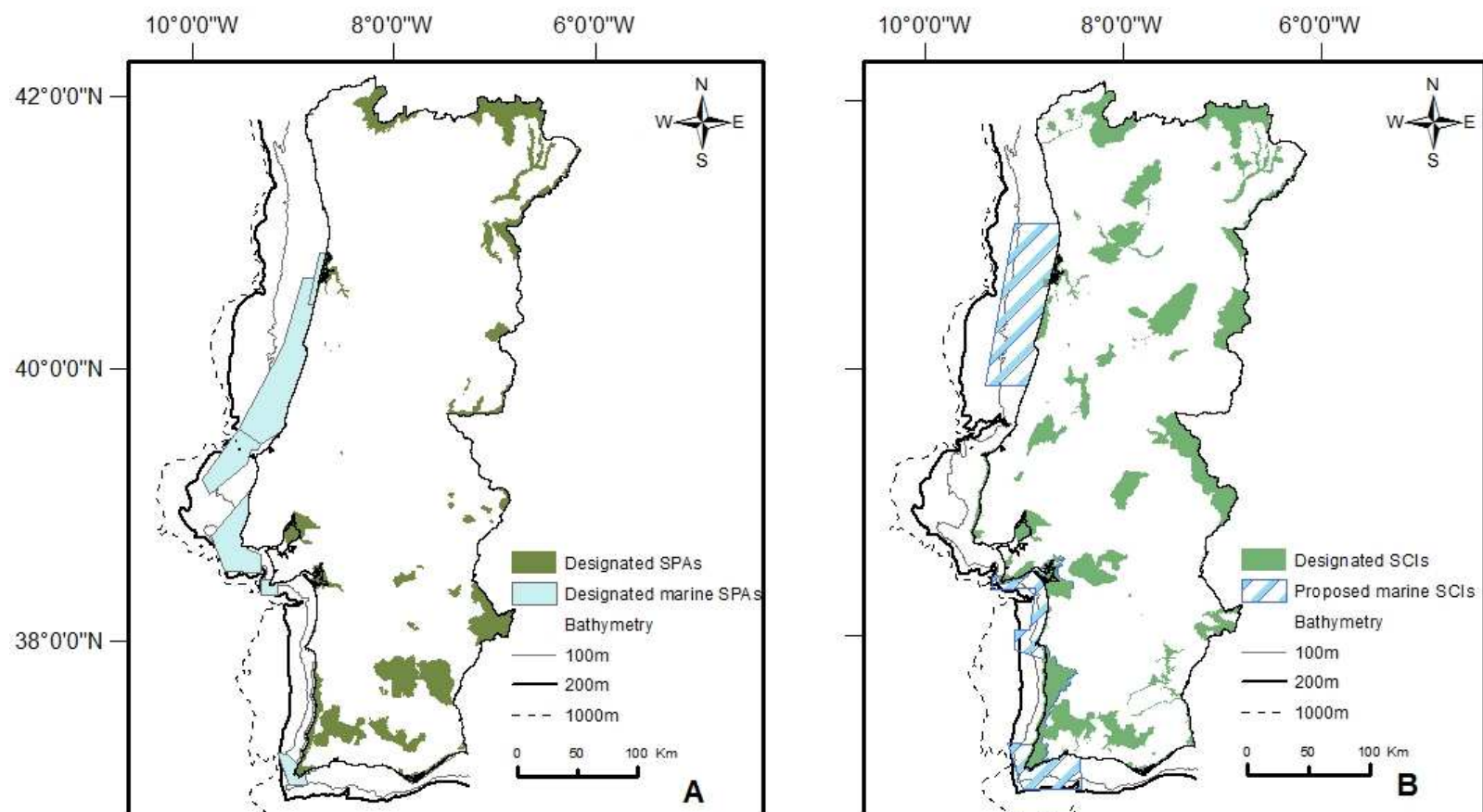


<b>Q5: Is the abundance of ecologically significant species (ESS) high in the subzone?</b>	<b>Rarity <sup>2</sup></b>	All components	Select ESS from species list Create 5 density classes for these species with values ranging from 1 to 5. Class 1 holds subzones without any ESS. If there are several ESS present in the subzone, create a different density class for each species separately and average the values afterwards.
<b>Q6: Is the species richness (SR) in the subzone high?</b>	<b>Aggregation/ fitness consequences <sup>1</sup></b>	All components	Determine the average SR for each subzone. Create 5 classes for SR ranging from 1 to 5.
<b>Q7: Is the abundance of habitat-forming species (HFS) high in the subzone?</b>	<b>Rarity <sup>2</sup></b>	macrobentos	Select HFS from species list Create 5 density classes for these species with values ranging from 1 to 5 (with an equal amount of subzones in each class). Class 1 holds subzones without any HFS. If there are several HFS present in the study area, then create a different density class for each species separately and average the values afterwards.
<b>Data Availability (DAV)</b>		All components	Determined by the number of samples (/observations) of each ecosystem component taken (/made) in each subzone. It is calculated for each ecosystem component and for all components together 1 = Low (L), 2 = Medium (M), 3 = High (H)
<b>Data Reliability (REL)</b>		All components	Based on the number of assessment questions that could be answered for each subzone in relation to the maximum amount of questions answered (number of questions answered per subzone)/(maximum number of questions answered). It is calculated for each ecosystem component and for all components together. 1 = Low (L), 2 = Medium (M), 3 = High (H)
<b>Biological Value (BV)</b>		All components	The biological value for each ecosystem component was determined by averaging the values for the different assessment questions. For the total BV, the individual BV available in each grid cell was averaged. Total BV 1 = Very Low (VL), 2 = Low (L), 3 = Medium (M), 4 = High (H), 5 = Very High (VH)

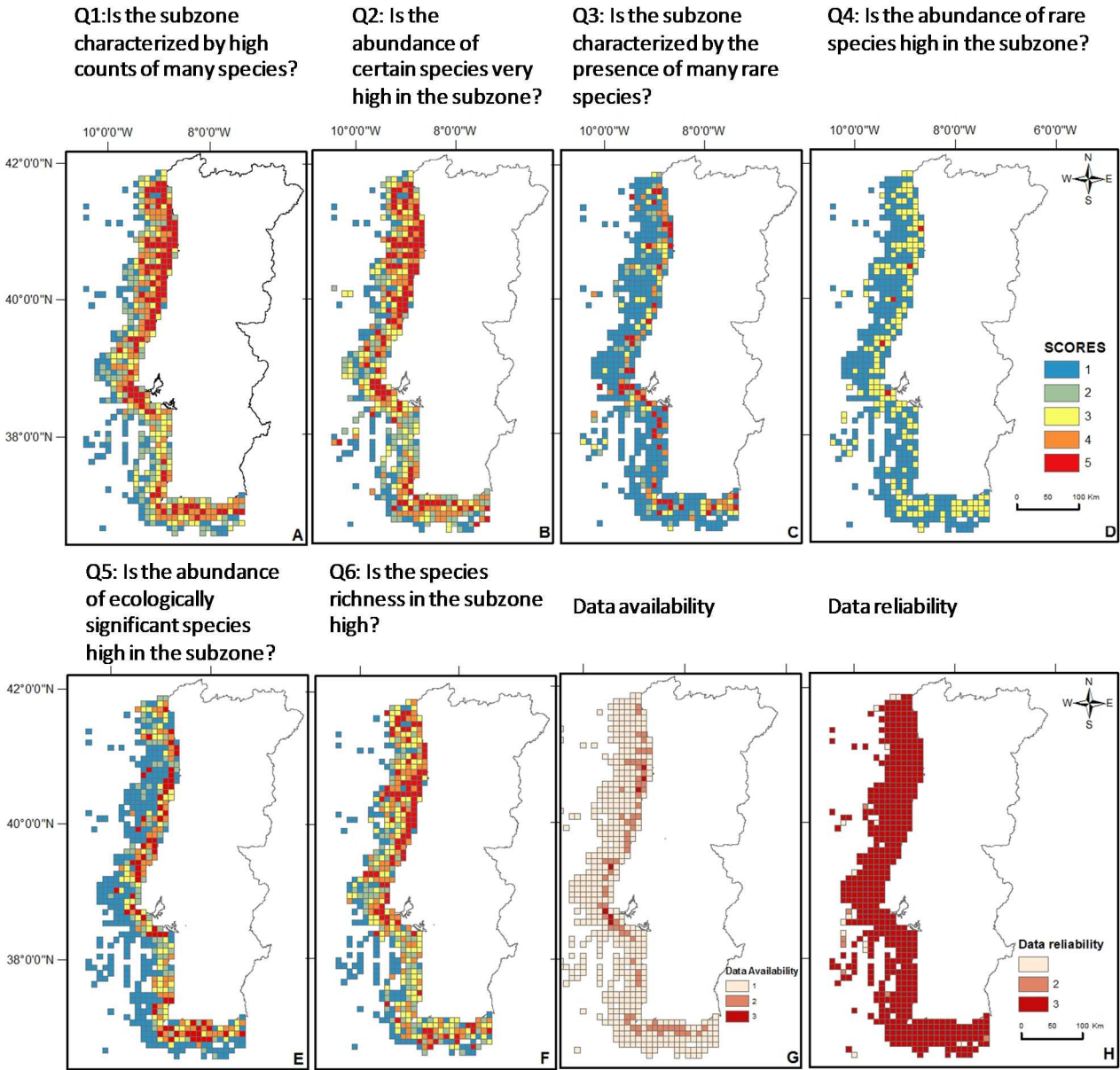
**Aggregation/ fitness consequences <sup>1</sup>** - Degree to which an area is a site where most individuals of a species are aggregated for some part of the year or a site which most individuals use for some important function in their life history or a site where some structural property or ecological process occurs with exceptionally high density / Degree to which an area is a site making a vital contribution to the fitness (=increased survival or reproduction) of the population or species present. DFO (2004) **Rarity <sup>2</sup>** - Degree to which an area is characterized by unique, rare or distinct features (landscapes/habitats/communities/species/ecological functions/geomorphological and/or hydrological characteristics) for which no alternatives exist. IUCN (1994), UNEP (2000), OSPAR (2003), DFO (2004)

### 5.8 Supplementary Information - Natura 2000 Network sites in continental Portugal

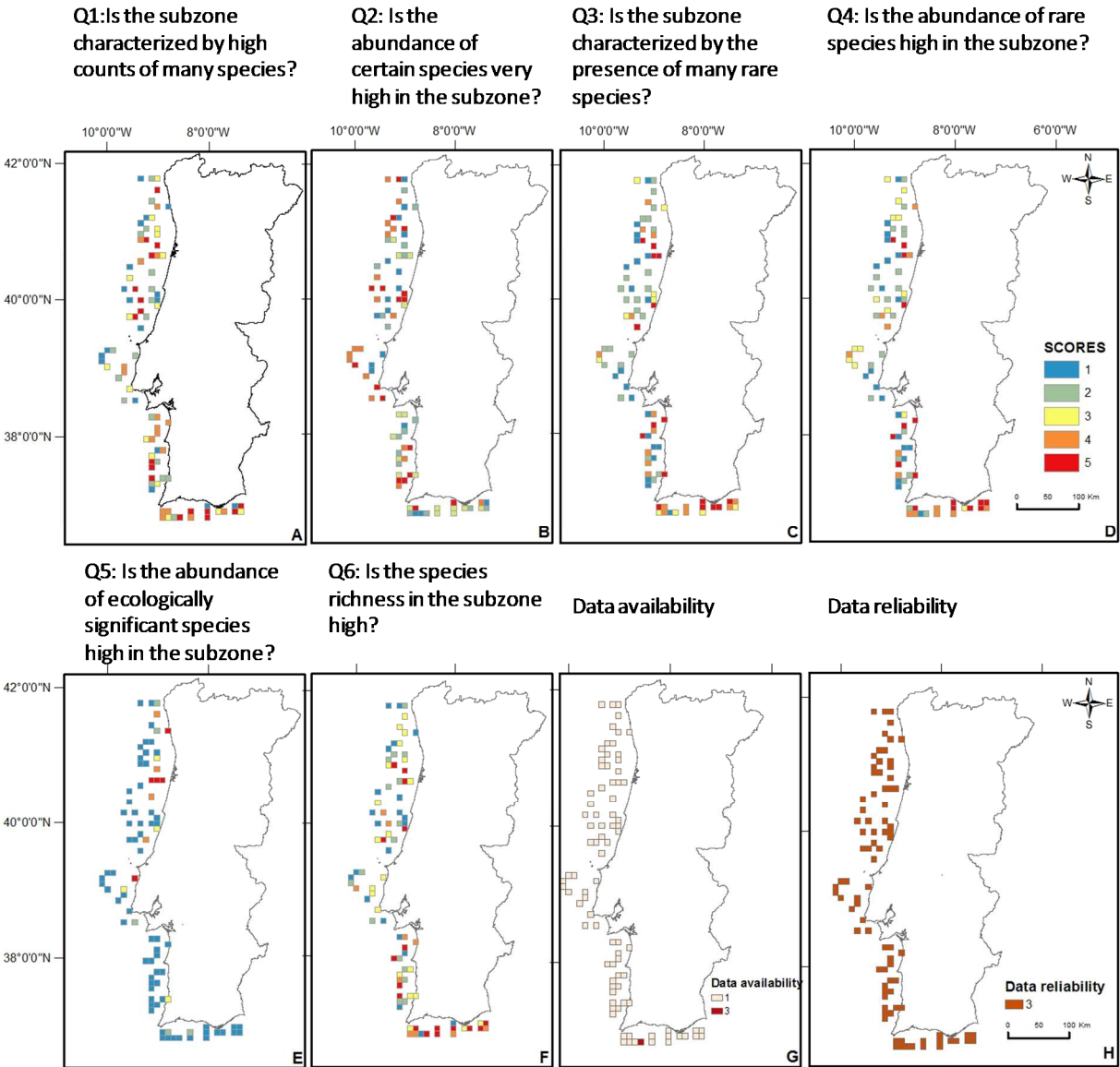
Figure S.1 **A** –Special Protection Areas (SPAs) **B** – Designated and proposed Sites of Community Importance (SCIs) (Fonte ICNF).



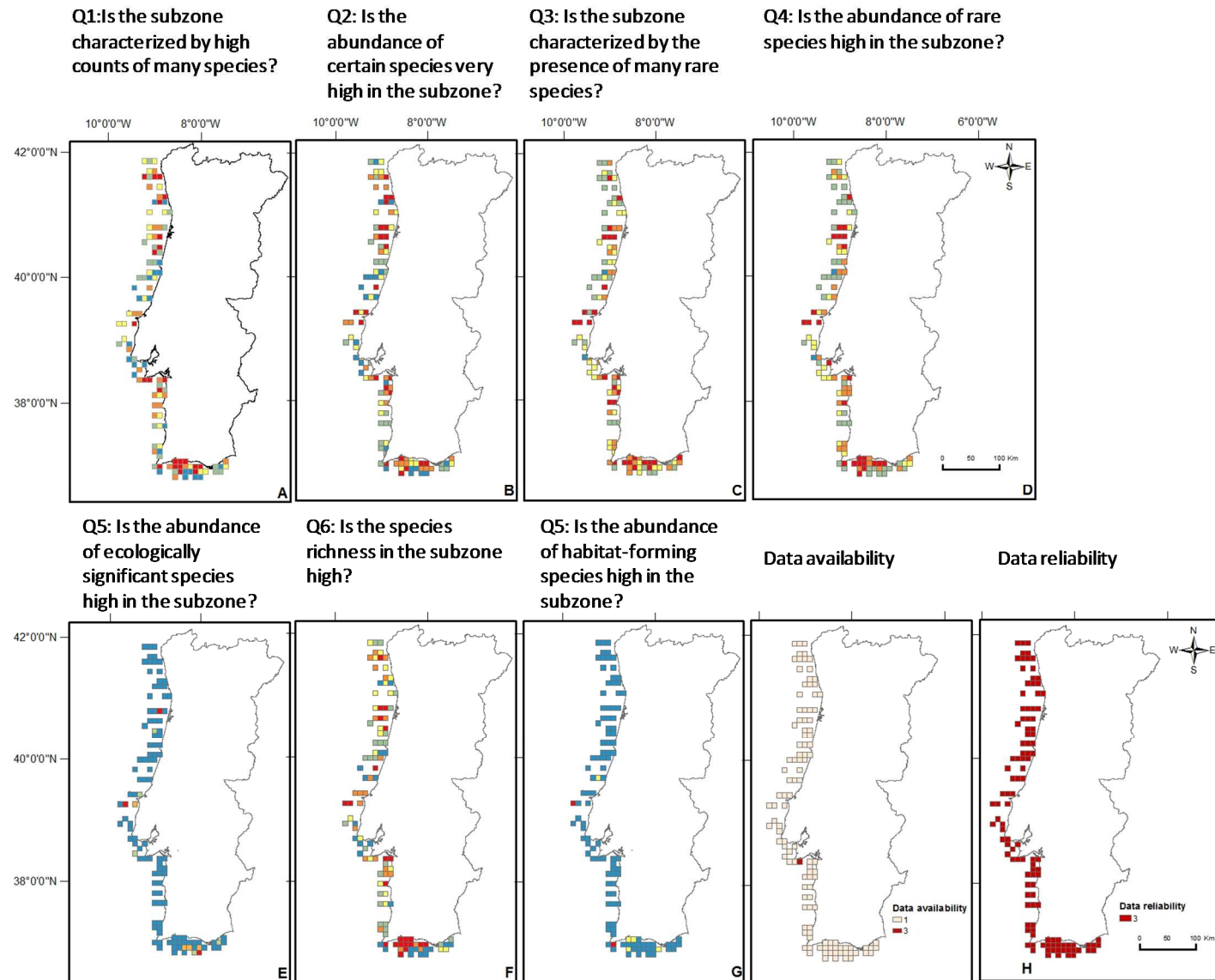
5.9 Supplementary Information – Results for the birds' component.



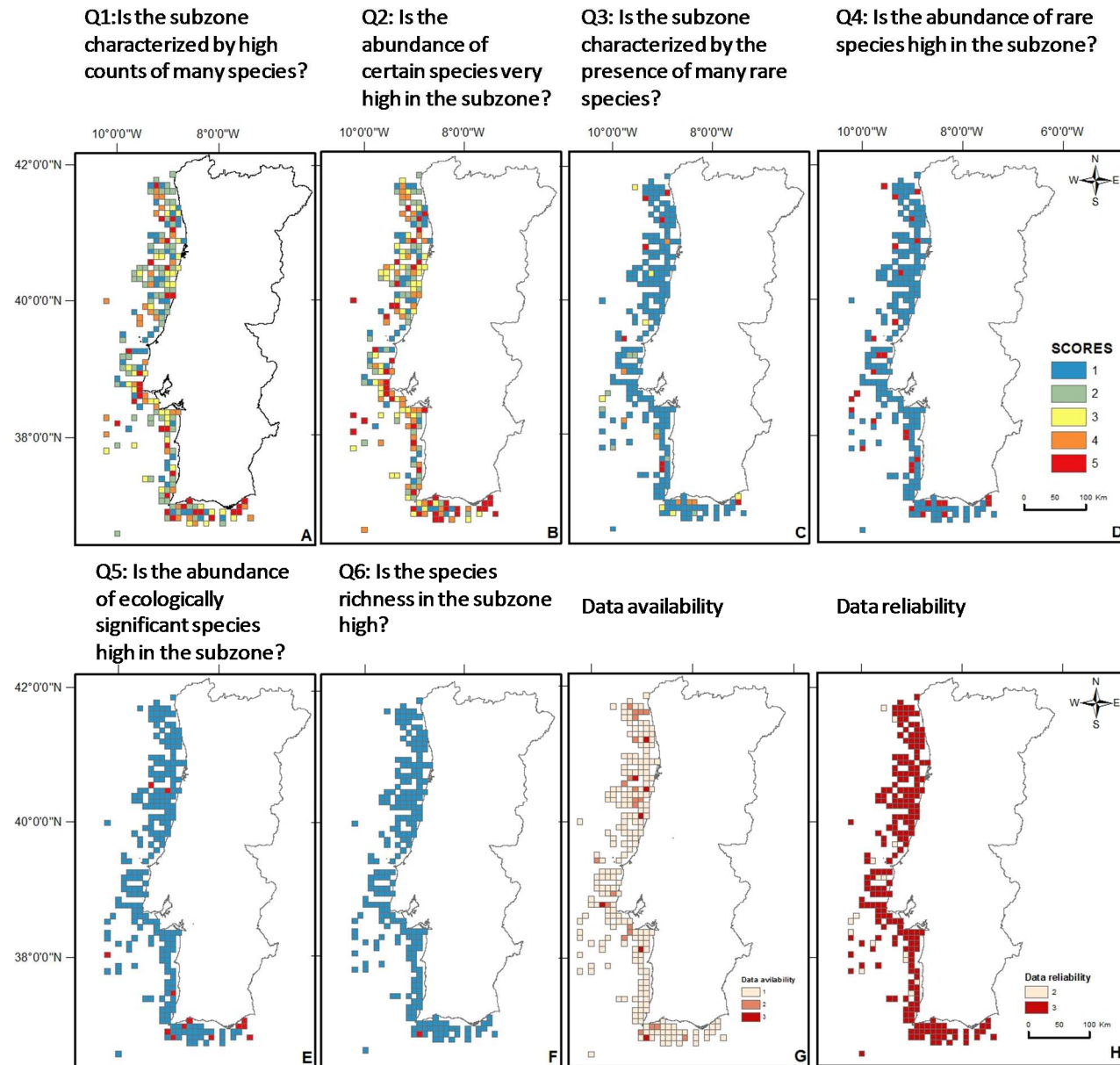
5.10 Supplementary Information – Results for the demersal fish component.



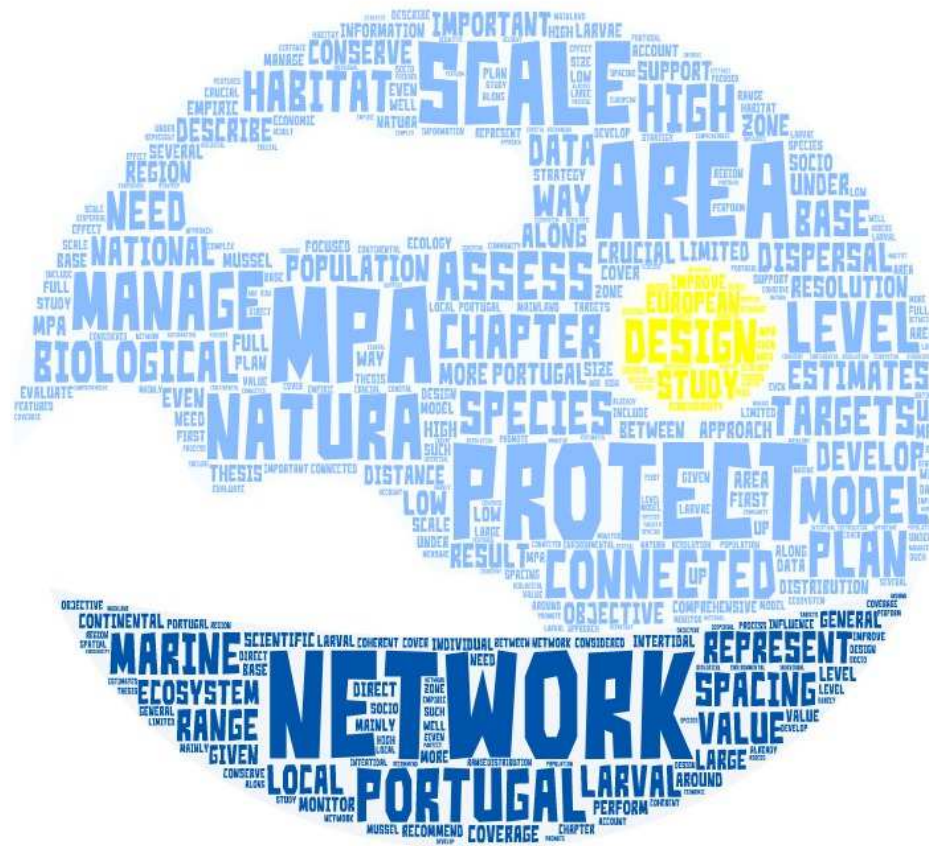
### 5.11 Supplementary Information - Results for the macrobenthos component.



### 5.12 Supplementary Information - Results for the marine mammals and turtles component.







## Chapter VI

## General Discussion and Future Directions

## 6.1 General Discussion

Portugal has committed to establish an ecologically coherent network of MPAs under several international agreements and national laws. Achieving ecological coherence is a complex task, and requires multiple approaches to analyse not only the location, size and spacing of protected sites already designated under the national government, but also inferring about new sites to enhance network effects. This process is complicated because individual MPAs have been set up with different goals, and under different monitoring, management and enforcement strategies.

Given the international deadline for the establishment of ecologically coherent networks of MPAs by 2020, the purpose of this thesis was to assist, promote and support this assessment along the Portuguese continental waters. Specific objectives included the development and application of different empirical methods focusing on important recognized criteria used to quantify ecological coherence: connectivity and representativity. Maintaining ecologically connected and representative networks of MPAs are two key conservation objectives to achieve biodiversity persistence (Margules and Pressey, 2000). A very recent study in the Mediterranean Sea, by Almeida Magris et al. (2018) provided evidence that biodiversity persistence is enhanced through achieving objectives for both representation and connectivity, in a multispecies context.

In this sense, this integrative quantitative approach did provide useful insights in this complex and contemporary issue. In addition, and as it is often the case at the end of a dissertation, it drew attention to several unanswered questions and emphasizes the need for a national strategy to set science-based targets which can maximize and enhance ecological coherence when designating new MPAs.

**Chapter I** highlighted the international pressure and progress in setting up coherent networks of MPAs so as to protect the structures and functions of marine systems, and promote economic and social benefits in an integrative manner. It specifically addressed how conservation planning, which is the base for the development of the networks, should be established in accordance with recognized biodiversity patterns and functions (Pressey 2004). It also showed that the criteria used to assess ecological coherence is consistent at the European level and comprehensively described in the literature. Even though a common set of criteria was proposed at the European level, in practice the targets may differ according to the scale of the assessment and the local environmental, socio-economic and political settings.



Threshold levels for an objective evaluation of coherence in a network of MPAs are yet to be set in Portugal. In this way, this thesis presented quantitative-based estimates on connectivity and representativity, and hopes to stimulate discussion among Portuguese scientists, stakeholders and managers on the design and monitoring of a national network of MPAs. This is indeed a very complex subject and there are few examples of studies which actually evaluate MPA networks coherence as a whole, considering all the settled criteria, and importantly, none of them rated an MPA network as coherent.

Regarding the Portuguese case, continental MPAs are limited to coastal waters, many of composing small extensions of terrestrial protected areas (fig. 1.3 in chapter I). The majority was created in the 70's and 80's (and reclassified afterwards), through ad-hoc actions addressing local and regional-scale goals and responding to local opportunities and restrictions. Therefore, they have been traditionally designed to conserve particular landscapes/ecosystems, from an aesthetic perspective and influenced by limited conservation efforts and minimization of socio-economic costs. In this regard, no "systematic planning" or network effects were considered in the overall MPAs' design and spacing. These areas contain, however, comprehensible management plans which account for their conservation objectives, which generally include the protection of habitats, species, cultural values and sustainable management of artisanal fisheries and economic activities. The average individual size of these classified areas is around 50 km<sup>2</sup>, ranging from 2.6 km<sup>2</sup> to 290 km<sup>2</sup>. However, fully protected areas (no-take zones) cover only 4.32 km<sup>2</sup> in Arrábida and 0.63 km<sup>2</sup> at Costa Sudoeste.

On the other hand, Natura 2000 sites in mainland Portugal have been created from around the year 2000 on, and have to comply with European Directives to create a network of sites to ensure the long-term survival of Europe's most valuable and threatened species and habitats. Therefore, Natura 2000 is different from the previously described national protected areas in the sense that all sites should be chosen according to scientific criteria to warrant the favourable conservation status under the entire European network.

The first result which stands out looking at the current MPA coverage in the mainland Portuguese coastal and ocean waters (considering both national classified sites and Natura 2000 sites) is its large extension. It represents 9.22% of the total Exclusive Economic Zone (EEZ) area of mainland Portugal, which is close to the 10% ambitious targets agreed by signatory states of the Convention on Biological Diversity. However, it is notorious the low percentage of strictly no-take zones, representing merely 0.0015% of the EEZ. Most importantly, the full protection coverage is mainly driven by a single MPA (Banco Gorringe Natura 2000 site of

Community Importance). This MPA alone covers 22 887.82 km<sup>2</sup>, almost three times more than all other MPAs summed together.

Most of the management plans for the Natura 2000 sites in mainland Portugal are still under discussion and await the approval by competent national authorities. It is important to keep in mind that Natura 2000 sites are designed and managed to provide protection for the selected habitats or species only, and do not warrant protection for the full array of biodiversity or territory. ICNF proposal for management guidelines suggests wide-ranging measures to *"minimize the disturbance, damage, destruction or removal of marine organisms and / or parts of habitat; prevent the release of harmful substances on site, regulate recreational and commercial fisheries; ensuring good environmental status of the site; condition the passage of ships in transit; regulate underwater tourism and encourage and support scientific research."*

So, rather than just looking to the overall protection percentage area, this guidelines need to turn into comprehensive and objective measures to guarantee that designated areas provide efficient protection on the ground. Nonetheless, despite recognized shortfalls in management implementation, the fact that these areas are already designated is a step forward to promote further improvement in protection. It is also crucial that management plans include key performance indicators covering not only environmental issues but also social and economic factors, which can considerably affect MPA performance as a protection tool (Charles and Wilson, 2008). In a recent study assessing management performance of MPAs in four countries in Northwest Europe, Portuguese MPAs showed a higher number of objectives and lower rates of objective achievement (Álvarez-Fernández et al. 2017).

So, the first real challenge dealing with an objective assessment of the ecological coherence of the continental Portuguese MPAs network has to do with this discrepancy in protected area designations, scale, objectives, and management and protection levels.

**Chapter II** proved that the model species *M. galloprovincialis* larval shell geochemistry is able to provide crucial information as an environmental and natal tag at an ecologically relevant spatial scale. The results showed that, during the period of the study, Arrábida MPA was the main source population supplying larvae to the other two regions, even though connectivity with Berlengas MPA was very limited. Also, that Arrábida MPA revealed high levels of self-recruitment within its bay, suggesting a retention zone for locally spawned larvae. Importantly, the dispersal distance for most of the recruits analysed was estimated to be less than 50 km away from the natal source.

Characterizing the extent of connectivity among marine populations and the factors influencing this exchange is fundamental to understand coastal species population dynamics (Cowen et al. 2006). This way, and although this work focus on a single-species quantitative measurement of connectivity it helped to identify the magnitude of larval dispersal within the study region, for an important ecosystem engineer component of rocky intertidal habitats. The rate of successful exchange of individuals within local populations of marine organisms drives population replenishment with repercussion for population dynamics. Therefore, realistic estimates of connectivity are crucial in spatial resource management to define the best size and range of reserve spacing (Botsford et al. 2001, Palumbi, 2003). Here, the results suggest that protected sites should be placed within around 50 km from each other to maximize benefits for mytilid marine larvae with potential large-scale dispersal among rocky intertidal areas.

Mytilid larvae have been described as a potentially long-dispersing species, with estimated dispersal distances reaching 100s of kilometres (Bayne, 1976). Although McQuaid and Phillips (2000) reported that the majority of recruits of *Mytilus galloprovincialis* in South Africa settled <5 km from the parent population, other studies (Gilg and Hilbish 2003, Kinlan and Gaines 2003, Becker et al. 2007, Smith et al. 2009, López-Duarte et al. 2012) also documented moderate dispersal distances (20–40 km) among open coast mussel populations.

Chapter II's results are also within the range of other targets set for MPA spacing within networks: Shanks et al. (2003) recommend a spacing of 10 to 20 km for species with typical pelagic larval durations, McLeod et al. (2009) proposed a general 15 to 20 km distance threshold between MPAs and Gaines et al. (2010) recommend 10 to 100 km distance. At a wider scale, the OSPAR Commission (OSPAR, 2007) recommends that spacing between nearshore MPAs should be less than 250 km and HELCOM (HELCOM, 2010) advises that 50 % of protected sites within the network should have more than 10 connections at 20 or 50 km distance. Finally, IUCN-WCPA (2008) suggests a spacing of 10 to 20 km, up to 50 to 100 km between individual MPAs and recommends variable spacing, as opposed to even spacing.

Although Chapter II was able to provide a detailed connectivity matrix, with mean dispersal directions and distances, it only represented a momentary and potentially transitory atlas of geochemical fingerprints. It illustrates a “snapshot” assessment of the local physical, chemical and oceanographic characteristics between June and July 2013, representing only one possible dispersal scenario and as a consequence, the results cannot be extrapolated to other areas or different seasons. Therefore, a broader temporal and spatial scale analysis of larval dispersal is needed, if we are to set management strategies related with MPA spacing at a national scale.

However, it is impossible to empirically measure the full range of spatial and temporal variability in larval dispersal for the whole area. Thus, predicting dispersal at greater scales and for multiple species and spawning sites is only feasible with the use of high resolution numerical models of ocean circulation coupled with biological parameters.

This way, empiric connectivity estimates, such as the results of chapter I, can support these models by fine-tuning and validation analysis, together with realistic estimates of species abundance and fertility along the study area.

**Chapter III** described the use of low altitude and high resolution drone imagery, 3D surface models and ground based observations as a powerful tool for surveying intertidal ecosystems. In monitoring intertidal areas, one of the major challenges concerns the spatial extent for which data needs to be collected, and the logistical constraints of broad scale ground surveys of small organisms existing in high densities. Adding to this, there are time constraints given the limited low tide interval. Aerial high resolution photographic surveys provided a fast assessment covering an ecological relevant spatial area, while presenting sufficient taxonomic resolution to capture fine-scale biotic features such as mussel beds. Importantly, this work was crucial to identify limitations concerning spectral characteristics, atmospheric conditions and resolution requisites.

Aerial images allowed for 3D investigation of the rocky substrate and determination of mussel coverage. The integration of the information of ground quadrat surveys allowed for an estimation of mussel density and mean size. The results also highlighted the relevance of wave exposure on the density and size distribution of mussel's populations along the Portuguese coast, especially during winter times. Higher mussel densities ( $n^{\circ}$  individuals/m<sup>2</sup> rock) were found at intermediate winter wave exposure indices, while smaller mussels were found at higher indices. Finally, these demographic parameters allowed the calculation of the reproductive output of the mussel population in the study area. Reproductive output has been described as key information to understand the persistence of spatially-structured populations within heterogeneous and patchy habitats (e.g. Trembl and Halpin 2012, Burgess et al. 2014). This way, our location-specific predictions can be used in metapopulation models dealing with the management of pivotal conservation areas.

Given time and logistics constraints, the developed protocol proved to be a very good option to obtain mussel density, size and reproductive output estimates to incorporate in the subsequent chapter. Nowadays, drone technology has the potential to modernize environmental science, providing adequate spatial or temporal resolution for ecology studies

at a relatively low cost (Anderson and Gaston 2013). The complete workflow, from flight planning, data acquisition, image processing and classification, and extrapolation, is described, so as to lay the groundwork for future routine applications in intertidal surveys. Our final predictive models of mussel abundance, size and reproductive output can provide important information to meet practical management and forecasting needs.

**Chapter IV** used the connectivity estimates of Chapter II to validate a biophysical model which included different population and larval biology scenarios as well as realistic assessments of mussel reproductive outputs calculated in Chapter III. Larval dispersal drivers are intrinsically a biophysical issue, given the interaction at various scales of physical processes (advection and diffusion properties of water circulation) and biological factors, such as reproductive output, growth, development, behaviour and mortality.

In this study, after testing the predictions from the biophysical model against the empirical data, there was a high level of convergence between the two independent estimates of larval dispersal at spatial scales below 40 km. Notably, this chapter explored the effect of accounting for uncertainty to improve cross-validation of independent estimates of marine population connectivity. In this way, the biophysical model described larvae trajectory in the central Portuguese west coast with very high certainty, and therefore it is expected to accurately predict mussel larvae dispersal in the remaining western Iberian margin. This opens the door to effectively conjugate the two techniques to describe a wider range of biological models and investigate demographic processes with promising applications in MPA management strategies.

The utilization of high-resolution biophysical models to study connectivity presents an obvious advantage over direct methods since it allows tracking of numerous virtual individuals with different life-traits, and over long spatial and temporal scales (Trembl et al. 2008). In this way, its outcomes are capable of illustrating the environmental variability crucial for robust selection of MPA networks (Cowen et al. 2006, Steneck et al. 2009, Katsanevakis et al. 2011).

If adapted to the species of interest, these estimates can help inform conservation priorities at different scales (Beger et al. 2010). Single or multiple species biophysical modelling of connectivity has already been used for marine-reserve optimization design in various habitats; e.g. in the Baltic Sea (Berglund et al. 2012), Mediterranean Sea (Andrello et al. 2013), Adriatic Sea (Bray et al. 2017), coastal British Columbia (D'Aloia et al. 2017), Indo-Pacific coral triangle (Trembl and Halpin, 2012), Tropical Pacific reefs (Trembl et al. 2008) and the Great Barrier Reef (Thomas et al. 2014). Recently, Krueck et al. (2017) used biophysical models to present a novel

approach to MPA design which promotes both population persistence and ensures effective fisheries recovery. Finally, biophysical models might help in anticipating how continental boundary currents are likely to change connectivity among a network of MPAs under future scenarios of climate change (Coleman et al. 2017).

Lastly, in **Chapter V**, biodiversity was valued along the Portuguese continental shelf using an ecological approach based on the intrinsic value incorporated in biodiversity per se, regardless of any human association. This approach was used as a means to assess representativity, and considered the extent to which high biological value were contained in the Natura 2000 network area. MPAs containing all community types and habitats, within well-connected networks should promote healthy ecological processes and support the persistence and resilience of ecosystems (Roberts et al. 2003).

Here, the marine biological valuation (MBV) protocol attempted to represent biodiversity features using species richness and composition, species rareness and presence of ecological important species using a wide taxonomic range of ecosystem components (seabirds, demersal fish, macrobenthos, marine mammals and sea turtles). The results showed four main hotspots of high biological value, which mostly overlapped with previously identified valuable areas for protection (Natura 2000 sites), particularly in the northern and central regions. However, there was a clear mismatch between high value areas, and low level of protection in the southern region. This type of analysis can be used to assist the expansion of new protected areas and/or develop spatial prioritization measures by drawing attention to subzones of biological importance. This biological value hotspot analysis was, however, dominated by the patterns describing the bird component. These long-lived marine predators can and have been used as biological indicators and sentinels to prioritize conservation efforts, given their wide range and overlapping distribution with other biological communities and anthropogenic stressors (e.g. Maxwell and Morgan, 2013).

In the context of ecological coherence, representativeness is usually assessed using targets measuring biogeographic regions and major habitats, as surrogates for biological features (e.g. OSPAR 2007). The rationale behind this approach is that species diversity generally increases with habitat diversity, thus, as more habitats are protected, more biodiversity is likely to be protected. Other surrogates include depth, distance to shore, seabed substrates, primary productivity and thermal fronts.

However, at a finer-scale study, such as this one, directly incorporating data on biodiversity distribution and abundance presents a more feasible approach for the assessment of

representativeness of biodiversity features. Nonetheless, caution is needed when interpreting relative biological value since such estimates are highly dependent on the quantity, quality, type of assessment questions selected and resolution of the biological distribution and abundance data. Therefore, it is also important to describe data reliability. Also, insuring representativeness of ecosystem features does not directly indicate their protection or promote their persistence (Pressey et al. 2015). In the end, and although most of the hotspot areas identified here already fall within the boundaries of Natura 2000 sites (except in the southern region), protection of the full array of ecosystem components might not be secured, since these sites were selected and will be managed based on a specific species - habitat Natura 2000 criteria.

In a nutshell, the main outcomes of this thesis are resumed in the table 6.1.

Table 6.1 Overview of thesis main outcomes and recommendations.

Criteria	Method	Study Area	Target Feature	Results	Recommendations
<b>Adequacy (Chapter I)</b>	Spatial coverage	Portuguese continental shelf waters	MPA network area coverage	Current MPA coverage represents 9.22% of the Exclusive Economic Zone of mainland Portugal; while no-take areas cover only 0.0015% of EEZ.	Approve and implement comprehensive management plans including key performance and management indicators.
<b>Connectivity (Chapter II)</b>	Trace elemental fingerprinting of the microchemistry of bivalve larval shells	Central Portuguese west coast	Rocky intertidal ecosystems and mussel reef populations as a model species with medium to large dispersal potential	Arrábida MPA was an important source population and showed high rates of self-recruitment but limited connectivity to the Berlengas MPA. Average dispersal distance was estimated to be less than 50 km away from the natal source, but reached more than 100 km in some cases.	Spacing amongst rocky shore protected areas should range between 50-100 km.
<b>Connectivity (Chapter III, IV)</b>	Low-altitude and high resolution aerial photographic surveys; Numerical biophysical model of mytilid larval dispersal - Regional Ocean Modelling System (ROMS) + Lagrangian biological model	Portuguese continental shelf waters	Demographic mussel estimates in rocky reefs; Broad scale mussel larvae dispersal	Winter wave exposure was significant in shaping mussel density, size and consequently, reproductive output. The predictions from the biophysical model were tested against the empirical data, with high level of convergence between the two independent estimates of larval dispersal at spatial scales below 40 km in the central Portuguese west coast with very high certainty.	Develop a spatially explicit population model to investigate effectiveness of the network for persistence of mobile species.
<b>Representativity (Chapter V)</b>	Marine biological valuation and hotspot approach	Portuguese continental shelf waters	Biodiversity components (Marine birds, marine mammals, macrobenthos, demersal fish)	Four main hotspots of high biological value were identified, and mostly fall within current and proposed Natura 2000 sites except in the southern region.	Expand protection zone in the southern continental shelf area.



## 6.2 Future directions and final remarks

Regarding the **Connectivity component**, and even though a great extent of critical work has been done investigating this section, no broad scale assessment of connectivity within the full network of MPAs has been completed. The development of 3-D biophysical hydrodynamic model required substantial time and associated costs for its development, calibration and validation, fundamental before running any simulations. In this sense, it will be crucial to use this calibrated model in the future to investigate seasonality, annual variation, and periodicity. It offers a powerful instrument to explore connectivity in the western Iberia system and test important scenarios of persistence of spatial-structured metapopulations within the network. The regulation and persistence of marine metapopulation in a network of MPAs depends on self-persistence (whether individuals reproduce enough in their lifetime to replace themselves) or network persistence (loops of connectivity among local populations in the network) (Botsford et al. 2001, Hastings and Botsford, 2006, White et al. 2010). Understanding the demographic connections between local populations will be essential to prioritise sectors of the coast to be protected and promote the persistence of metapopulations, support their recovery from disturbance and grant benefits for both conservation and fisheries management (Guichard et al. 2004, Almany et al. 2009, Green et al. 2014). As an exemple, Jones et al. (2007) advocated prioritizing the protection of spawning aggregation sites, isolated sites and source populations, for the persistence of coral reef metapopulations.

On the topic of **Marine Biological Valuation**, the protocol should be discussed amongst environmental researchers, stakeholders and policy makers for future refinement in the choice of assessment questions. It should also be improved with up-to-date information on the national distribution and abundance of marine organisms and habitats and include spatial data on the distribution and abundance of other important marine ecosystem components, such as pelagic fish, phytoplankton and zooplankton. Finally, the method should be applied at a smaller spatial scale, including different habitats, such as transitional waters, seagrass and kelp beds, saltmarshes, rocky and sandy shores to improve the valuation at the intertidal and shallow subtidal coastal zones.

In general terms, and with reference to future broad assessments of ecological-coherence in Portuguese MPAs, the first and most important (and probably the most difficult) task will be to agree on scientific-grounded threshold levels for the criteria underpinning ecological coherence.

Consequently, targets should be set for the general criteria of connectivity, adequacy, representativeness and replication, and adapted to the scale of the assessment.

Ultimately, these targets should reflect exhaustive meteorological, oceanographic and biological research for adequate MPA network design, location, size and spacing (Green et al. 2014). Also, they should take into consideration the human dimension (socio-economic setting) so that knowledge can be directly linked to action (Fox et al. 2012).

It is, therefore, a complex and multidisciplinary task due to the complex networks of biotic/abiotic interactions, and socio-economic factors, which define marine socio-ecological systems.

Moreover, there are still a number of scientific knowledge gaps and logistical constraints, mainly driven by:

- The lack of broad scale available datasets at a national level on biodiversity and habitats, anthropogenic stressors and threatened features. In many areas, biological and geological sampling coverage is often spatially inconsistent and temporally unrepresentative, yielding unsatisfactory information in terms of species distribution and abundance or habitat continuity.
- The need of improved information on comprehensible management plans and protection levels across MPAs.
- The need to address transboundary issues, local socio-economic setting and the accessibility of incentives to plan, manage, monitor and finance those areas.

Nonetheless, the complexity and the lack of complete information should not hinder our attempt to deal and resolve environmental and socio-economic problems at sea (Tallis and Lubchenco, 2014). The prompt development of technology and computational abilities, and emergent data availability, mainly on GIS records on the spatial distribution of conservation features, species, habitats and anthropogenic pressures, is likely to improve management initiatives backed by sound scientific evidence.

This way, the best available scientific information should be used to discuss and define measurable targets for an objective evaluation of the current network performance and for the development of competent expansion and management plans. Table 6.2 resumes future research needs and required threshold levels for the assessment of ecological coherence of networks of MPAs at a national level, based on the work developed in this thesis and the experiences described for other European seas.

Table 6.2 – Future research needs concerning threshold levels for the assessment of ecological coherence of networks of MPAs at a national level.

Criteria	Rationale	Targets to be set at a national level:
<b>Adequacy</b>	Safeguard the ecological viability and integrity of marine species and communities by protecting sufficient proportion of features to secure their long-term persistence and resilience.	<ul style="list-style-type: none"> <li>- total area to be protected within the network.</li> <li>- optimal size ranges for individual MPAs</li> <li>- spacing between adjacent MPAs within the network</li> <li>- minimum size for no-take zones</li> <li>- implement adequate management plans including key performance indicators.</li> <li>- secure accessibility of incentives to enforce, monitor and finance those areas</li> </ul>
<b>Connectivity</b>	Guarantee MPA spacing within the network which allows for sufficient exchange of eggs, propagules, larvae recruits, juveniles or adults, at the species' range.	<ul style="list-style-type: none"> <li>- measures of dispersal distances for different species</li> <li>- optimal size ranges for individual MPAs</li> <li>- spacing between adjacent MPAs within the network</li> <li>- distance between landscape patches (habitat continuity) and areas of ecological importance (nursery, spawning)</li> </ul>
<b>Representativity</b>	Include the full range of ecosystems, habitats and the biotic diversity, ecological processes and environmental gradients within the network.	<ul style="list-style-type: none"> <li>- % cover of marine subregions and/seascapes</li> <li>- % cover of EUNIS level 3 habitats</li> <li>- % cover of depth zones</li> <li>- % cover of threatened and/or rare habitats and species</li> <li>- % cover of areas of high biological value</li> </ul>
<b>Replication</b>	Protect a sufficient number of species and habitats to safeguard ecological processes within the network and protect them from risks affecting individual MPAs.	<ul style="list-style-type: none"> <li>- minimum number of replicates for selected marine landscapes</li> <li>- minimum number of replicates for benthic marine habitats</li> <li>- minimum number of replicates for vulnerable/rare habitats and species</li> </ul>

### 6.3 References

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